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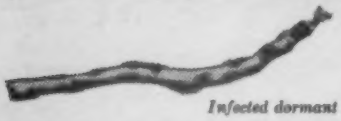 *Infected dormant terminal.*	Watch for infected terminals . . . a sure sign that powdery mildew has overwintered in buds and on twigs, and will become active when buds break. Start application of KARATHANE in the pre-pink period.
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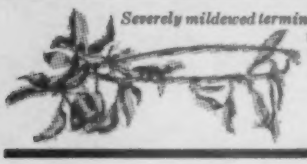 *Severely mildewed terminals and foliage resulting from uncontrolled primary and secondary infection.*	This type of foliage damage and overwintering of mildew can be prevented only by continuing KARATHANE applications through the period of terminal growth. Since mildew fungi are "waxy" and hard to wet, include an efficient spreader-sticker, such as TRITON B-1956 in your sprays.

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THE EFFECTS OF MELOIDOGYNE INCOGNITA AND M. HAPLA ON THE GROWTH OF KENLAND RED CLOVER AND ATLANTIC ALFALFA

Richard A. Chapman

Plant Pathologist.

Accepted for publication Sept. 1, 1959. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

SUMMARY

The growth, measured by top dry weight production, of Kenland red clover was seriously reduced by *Meloidogyne incognita* and *M. hapla*. The reduction was produced to a geater degree and more rapidly by *M. incognita* than by *M. hapla*. The growth of Atlantic alfalfa was seriously reduced by *M. hapla*, but not by *M. incognita*. *M. hapla* affected both plants to the same degree.

Trifolium pratense L. (red clover) and *Medicago sativa* L. (alfalfa) are hosts of root-knot nematodes. McGlohon and Baxter (6) determined the susceptibility, based on root-knot indices, of 18 species of *Trifolium*, including *T. pratense*, to *Meloidogyne incognita acrita* Chitwood, and found them all susceptible. On the same basis, Allison (1) determined the relative susceptibility of several varieties each of red clover and alfalfa to *M. hapla* Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid & White) Chitwood in addition to *M. incognita acrita*. Red clover was very susceptible to all 5 nematodes, whereas alfalfa was very susceptible to *M. hapla* and moderately susceptible to the others. Individual plants of certain varieties and accessions of alfalfa were resistant to all 5 nematodes. Bain (2, 3) found individual plants of certain accessions of red clover that were resistant to *M. arenaria* or *M. javanica* or *M. incognita acrita*. Reynolds (8) found that most of several varieties of alfalfa were more susceptible to *M. javanica* than to *M. incognita acrita*. One variety, African, was resistant to both species, and the variety Chilean was resistant to *M. incognita acrita*. Root-knot indices were the basis for determining relative susceptibility. On the same basis, Stanford et al (9) determined the relative susceptibility to *M. hapla* of many varieties and Foreign Plant Introductions of alfalfa and species of *Medicago*. Individual plants of the varieties Vernal and Hilmar were resistant.

McGuire et al (7) and Walters and Slack (10) found that the severity of fusarium wilt, caused by *Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atk.) Snyd. & Hans., in Buffalo alfalfa increased as the root-knot index increased. Root-knotting was caused by different species of *Meloidogyne* in increasing order as follows: *M. incognita acrita*, *M. arenaria* and *M. incognita*, *M. javanica*, and *M. hapla*.

The present investigation was undertaken to determine the effect of *M. hapla* and *M. incognita* on the growth of Kenland red clover and Atlantic alfalfa. An abstract of the results has been published (5).

Materials and methods.—Larvae of the 2 nematodes were obtained from colonies reared from single egg masses on *Nicotiana tabacum* L. (tobacco, variety Ky 58). One-half-gallon glazed crocks of steamed soil were each infested with 3000 larvae in Experiment I, and with 1000 larvae in Experiment II. The infestation technique has been described (4). An essentially larva-free suspension of associated microorganisms was made by combining suspensions of the 2 nematodes and repeatedly pouring the mixed suspension through a U.S. No. 325 sieve. Each crock, including controls, received 50 ml of this preparation.

Seeds of red clover and alfalfa were sown at the time the soil was infested. After about 2 weeks the plants were thinned to 4 and 5 per crock for red clover and alfalfa, respectively. Growth was measured by determining the dry weights of frequent harvests, made by cutting off the top growth 2 in. above the surface of the soil.

Each treatment was replicated 4 times, and the crocks were arranged in contiguous randomized blocks, 1 each for red clover and alfalfa, on a greenhouse bench.

Results.—The growth of red clover was decreased severely by both nematodes (Table 1). Experiment I was continued for 152 days (6 harvests), and Experiment II was continued for 317 days (11 harvests). All plants in crocks infested with *M. incognita* were dead in 105 and 154 days in Experiments I and II, respectively. In the same order, at the conclusion of the experiments there were 2 and 5 living plants in crocks infested with *M. hapla*. Roots of all inoculated plants were severely galled, and most of them were rotted. Injury was produced more rapidly by *M. incognita* than by *M. hapla*, and it was produced more rapidly by both nematodes in Experiment I than in Experiment II. Growth of the control plants was more rapid in Experiment II than in Experiment I.

The growth of alfalfa was decreased severely by *M. hapla*, but not by *M. incognita* (Table 1). At the conclusion of the experiments there were 3 and 6 living plants in crocks infested with *M. hapla* in Experiments I (175 days, 7 harvests) and II (381 days, 14 harvests), respectively. In the same order there were 18 and 16 living plants in crocks infested with *M. incognita*. In the last 2 harvests in Experiment II the yields were significantly lower from plants in crocks

Table 1.—Effects of *M. hapla* and *M. incognita* on the growth of Kenland red clover and Atlantic alfalfa in two experiments (I and II).

	Dry weight (g)			
	Red clover		Alfalfa	
Treatment	I	II	I	II
M. hapla	1.92**	16.43*	2.37**	16.33*
M. incognita	0.99**	4.50**	5.54	26.11
Control	4.25	27.60	5.61	27.95

* Difference from control significant at 5% level.
** Difference from control significant at 1% level.

infested with *M. incognita* than from control plants. Roots of all inoculated plants were galled, and those infested with *M. hapla* were rotted. Injury by *M. hapla* was produced more rapidly in Experiment I than in Experiment II. Growth of the control plants was more rapid in Experiment II than in Experiment I.

Discussion.—*M. hapla* reduced the growth of both plants by nearly identical percentages: red clover by 55 and 40% and alfalfa by 58 and 42% in Experiments I and II, respectively. The effects of *M. incognita* on the growth of plants were consistent in the 2 experiments, but not the same for both plants. The growth of red clover was reduced 77 and 83%, and that of alfalfa was reduced 2 and 7% in Experiments I and II, respectively.

Both reduction in vigor and death of plants were factors in the reduction of growth. Decreases in yields of individual harvests were significant before plant stands were reduced.

Reduction in growth was greater and occurred more quickly when *M. hapla* was used at the higher inoculum level. The over-all results with *M. incognita* were the same at both inoculum levels, but, where reduction in growth occurred, the effect was achieved more quickly at the higher inoculum level.

Both nematodes reproduced on both plants. Growth of red clover was reduced more rapidly by *M. incognita* than by *M. hapla*, whereas the growth of alfalfa was reduced much more rapidly by *M. hapla* than by *M. incognita*. Injury to alfalfa by *M. incognita* was becoming apparent in the last 2 harvests of Experiment II.

These results parallel those of others (1, 7, 8, 10) in which the relative susceptibilities of red clover and alfalfa to *M. hapla* and *M. incognita* and the closely related *M. incognita acrita* were based on root-knot indices.—Kentucky Agricultural Experiment Station, Lexington.

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THE CAUSAL AGENT OF BACTERIAL BROWN ROT OF CYPRIPEDIUM ORCHIDS

Donald D. Sutton, Peter A. Ark, and Mortimer P. Starr

Respectively, Predoctoral Fellow of the National Science Foundation, 1955–57. Present address: Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey; Professor of Plant Pathology, University of California, Berkeley; Professor of Bacteriology, University of California, Davis.

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SUMMARY

Erwinia cypripedii, the causal agent of bacterial brown rot of Cypripedium orchids, is a small, peritrichous Gram-negative bacterium. The organism is capable of metabolizing a wide range of sugars and other biochemical substrates. The distinctive biological characteristics of *E. cypripedii* are compared with those of other species of *Erwinia*. Unlike *E. carotovora*, *E. cypripedii* is non-pectolytic and has other attributes that would exclude it from the soft rot group of *Erwinia* species. *E. cypripedii* seems to fall more appropriately in the "true *Erwinia*" group. Although it is quite unlike the type species *E. amylovora*, the pathogen shows some degree of relatedness to *E. nigrifluens*.

Brown rot of tropical orchids was well described by Hori (8) in 1912 as a serious disease of several species of *Cypripedium* and *Phalaenopsis* grown in hothouses in Tokyo. The same disease occurred in orchids grown in their natural habitat on Formosa. Hori isolated identical bacteria from many cases of the disease and proved them pathogenic. He considered the pathogen similar to *Bacillus carotovorus* Jones (9) but morphologically and physiologically distinct, and proposed the name *Bacillus cypripedii*. Both of these species were later placed in the genus *Erwinia* (6).

Neither the disease nor the pathogen was recognizably reported again until 1950, when brown rot of Cypripedium orchids reached serious proportions in several California nurseries. The symptoms agreed in every detail with those described by Hori. The disease as it appeared in 1950, and its control, have been described elsewhere (1, 2).

This paper presents the biological characteristics of the causal agent, *Erwinia cypripedii*. The results are presented in as full detail as the description of a new species, for the following reasons:

1) Our earlier reports (1, 2) dealt with immediate need for recognition and control of the disease, though further study was needed to clarify the taxonomic position of the pathogen. The present clarification

stems from subsequent comparative studies of *E. cypripedii* and other species of the genus *Erwinia* (13).

2) The original description by Hori lacks many of the biochemical characteristics now employed in comparative study of pathogenic bacteria.

3) Revision of the genus *Erwinia* for the fifth edition of *Bergey's Manual of Determinative Bacteriology* (4) relegated *E. cypripedii* to the dubious position of a "possible synonym" of *E. carotovora*. The implication of synonymy was probably influenced by the report (10) of a soft rot pathogen from the Phalaenopsis orchid that could attack Cypripedium orchid after artificial infection. The pathogen was biochemically indistinguishable from *E. carotovora*.

4) *E. cypripedii* is not recognized or even mentioned in the current seventh edition of *Bergey's Manual* (5).

Bacteriological studies.—Fourteen cultures, comprising eleven isolates from typical brown rot of *Cypripedium* spp. and three reisolates from artificial infection, were studied by bacteriological determinative methods after rigorous checks to assure purity and pathogenicity. Many of the methods used have been described elsewhere (7, 12); with the exceptions stated, test cultures were incubated at 31°C. Fifteen cultures of *E. carotovora* from calla (3), potato, and pepper were studied at the same time.

Morphology.—The pathogen is a small rod-shaped bacterium with rounded ends; cells occur singly, rarely in pairs. Cells were actively motile in wet mounts; motility was also demonstrable as diffuse growth in semisolid (0.4%) agar nutrient media. Gray's flagella stain showed 4–9 peritrichous flagella per cell. Endospores were not seen in stained smears. Cells from one-day-old cultures in beef-peptone broth stained Gram-negative (Hucker's modification) although retention of stain by granules resulted in a stippled appearance. Cells in stained smears measured 0.5–0.7×0.7–1.4 μ.

Cultural characteristics.—On Bacto nutrient agar, small (1.5–2.2 mm), round, convex colonies were formed in 24 hours, with these characteristics: entire edge, smooth glistening surface, colorless. In 48 hours the colonies had grown little but had become umbilicate with undulate edges and irregular surfaces. The centers of colonies were especially depressed in crowded areas.

On PDP (infusion from 30 g potato per 100 ml water, plus 2% glucose, 1% peptone, 2% agar), round, convex colonies similar to those seen on nutrient agar were formed in 24 hours. In 48 hours at 31°C the colonies had grown little but had become flat with concentric ridges. The cultures had a strong acid odor and failed to grow on transfer to fresh media.

On YDC (1% yeast extract, 2% glucose, 2% $CaCO_3$, 2% agar), round, pulvinate colonies formed in 24 hours, with these characteristics: entire edge, smooth glistening surface, viscid consistency, colorless to light yellow-brown, 2.0–3.5 mm diameter. In 48 hours the colonies had grown to 5 mm diameter; adjacent colonies coalesced readily. Calcium carbonate was dissolved beneath the colonies. In 72 hours the colonies and surrounding areas were yellow-brown to red-brown; carbonate was dissolved extensively beyond the colonies. After 18 days' growth, colonies were irregular with papillae and concentric and radial ridges. The colonies and medium were orange-brown; dissolution of carbonate was followed by redeposition under some colonies. In contrast to the rapid death on PDP agar, colonies on YDC yielded viable cells after 18 days' growth.

On Bacto EMB agar (containing eosin, methylene blue, lactose, and sucrose), round, pulvinate colonies with purple centers formed in 24 hours. The smooth glistening surface had the green metallic sheen characteristic of strongly acidogenic organisms such as *Escherichia coli*.

Growth in nutrient broth (beef extract-peptone) was apparent throughout the culture tube in less than 18 hours. Continued growth yielded a flaky sediment; sometimes a ring formed at the top of the medium, but not a pellicle.

Physiological requirements.—The optimal temperature for rapid growth was tested in nutrient broth at 24, 27, 31, and 37°C. Ten cultures grew best at 31, three at 27, and one culture grew equally well at 27 or 31°C.

Moderate salt tolerance was shown by good growth in nutrient broth containing up to 7% sodium chloride, but not 9%.

The simple nutritional requirements of the organism were shown by abundant growth in a glucose-mineral salts medium (11). Growth was good in this medium whether incubated in air or under nitrogen. Growth occurred throughout shake cultures in yeast extract-glucose agar, but only on the surface of shake cultures in yeast extract agar.

Biochemical reactions.—1) *Pectolytic activity.*—The ability to liquefy gels of sodium ammonium pectate is characteristic of the pectolytic or soft rot pathogens of the genus *Erwinia* (12, 13). *E. cypripedii* was unable to hydrolyze and liquefy pectate. This negative finding, re-examined later, is discussed below.

2) *Action on carbohydrates.*—*E. cypripedii* displayed a wide range of fermentative ability when grown in the mineral salts medium (11) with various carbohydrates as single carbon sources. The reactions were tested in Durham tubes with a mixed indicator (bromthymol blue and chlorphenol red) and 1% filter-sterilized sugars. Twelve cultures formed large amounts of acid and gas from the pentoses L-arabinose, xylose, rhamnose; the hexoses glucose, fructose, galactose, mannose; the disaccharides sucrose, maltose, lactose, cellobiose and trehalose; salicin, mannitol and inositol. Two cultures formed acid without gas from the same sugars. All cultures formed acid without gas from melibiose, dextrin, inulin, ethanol, and glycerol. Fermentation of lactose, melibiose, and inulin was delayed, appearing after 3 weeks' incubation. No growth, or only slight growth without detectable acid production, occurred in raffinose, melezitose, esculin, sorbitol, dulcitol, or adonitol.

Tests for fermentation of sugars were repeated with four representative cultures, using 0.5% BBL Trypti-

case as the basal medium. The results confirmed those seen in the mineral salts basal medium, with two exceptions. Raffinose was fermented and lactose was fermented more promptly in the Trypticase medium. Some additional substrates were tested with four cultures—ribose, gluconate, glucuronate, galacturonate and mucate were fermented. D-arabinose and alpha-methylglucoside were not fermented.

Growth of all isolates in bromcresol purple milk yielded a weak acid reaction in 24 hours, indicative of glucose fermentation. Acid coagulation of casein, indicative of lactose fermentation, did not occur until 3 weeks later. Peptonization did not take place in 6 weeks.

Positive Methyl Red reactions indicated formation of large amounts of acids from glucose. Acetylmethylcarbinol was not formed; negative V-P tests (7) were obtained after 1, 2, 6, and 22 days' growth in Bacto MR-VP medium.

Action on starch was tested on plates of nutrient agar containing 2% Swan potato starch by flooding the plates with Lugol's iodine after 10 days' growth. Violet zones against a dense blue background indicated partial hydrolysis of starch by *E. cypripedii*. Diastatic activity was not demonstrable when soluble starch—the usual substrate—was employed.

3) *Utilization of organic acids.*—In the mineral salts basal medium (11), growth with alkaline reactions indicated utilization of acetate, lactate, and tartrate. Further metabolism of tartrate yielded acid and gas, an unusual reaction. Growth with an alkaline reaction on Simmons' citrate agar slants showed utilization of citrate as the sole carbon substrate. Four representative cultures were also grown on slants of Simmons' medium containing galacturonate, malonate, or formate instead of citrate; alkaline reactions showed utilization of these substrates.

4) *Metabolism of nitrogenous substrates.*—Stab inoculations in nutrient gelatin showed slow filiform growth after 24 hours at 24°C. Surface liquefaction was observed only after 18 days' growth. The zone of liquefaction became saccate after 25 days and was complete within 90 days.

Formation of indol from tryptophane could not be detected with Kovac's reagent after 1, 2, or 16 days' growth in Bacto Tryptone.

Hydrogen sulfide production was detected with lead acetate papers suspended over broth cultures in BBL Thiotone, a peptone rich in sulfur-containing amino acids. This process was not demonstrable with peptone-lead acetate agar or Bacto triple sugar iron agar.

Tyrosinase activity was manifested by formation of a red-brown pigment during 3 weeks' growth in a synthetic tyrosine medium (12).

Reduction of nitrate to nitrite was demonstrated in 12 cultures after one and four days' growth in Bacto nitrate broth. Sulfanilic acid and alpha-naphthylamine were the reagents used (7). One culture yielded negative tests for nitrite; another showed only a weak reaction after one day and was negative after 4 days' growth. It may be significant that these two cultures were those that also failed to form gas during fermentation of sugars.

Re-examination of key characteristics.—Many of the biochemical reactions presented above were confirmed in a later, more extensive comparative study of the genus *Erwinia* (13). The cultures studied included recent isolates as well as reference cultures dating back as far as 1902. The collection of 58 isolates comprised 10 named species of the soft rot group, 5 species of the "true *Erwinia*" group, and 10 named species of *Erwinia* with uncertain taxonomic affinities; the last mentioned group included two typical and two atypical (anaerogenic) isolates of *E. cypripedii*. In this latter study, cultures were incubated at 28°C. The results that amend or clarify the earlier observations are discussed below.

1) *Test for pectolytic activity.*—The inability of *E. cypripedii* to hydrolyze pectic acid was confirmed with a sensitive modification of the pectate gel medium. A hot 3% pectate sol, prepared as originally described (12), was diluted with two volumes of hot yeast extract solution (0.5%), tubed, and autoclaved. *E. cypripedii*, heavily inoculated by stabbing a loopful of cells from a slant into the tubes of gel, grew with no visible action on pectate during 28 days' incubation.

On the other hand, liquefaction of the modified pectate gel was found to be a sensitive and reliable index of the pectolytic activity of *E. carotovora* and related soft rot organisms. All soft rot cultures examined, even old reference cultures with diminished virulence to plant tissue, produced extensive liquefaction of the gel within 24 hours.

2) *Nitrate reduction.*—The inability of two isolates of *E. cypripedii* to yield positive tests for nitrate reduction was examined further. It seemed reasonable to determine whether the organisms could grow in a synthetic medium containing nitrate as the only source of nitrogen. Since the nitrogen in cell protein is about at the oxidation-reduction level of $-NH_2$, growth in this medium would indicate reduction of nitrate. The medium used contained 1 g potassium nitrate, 5 g glucose, 0.1 mg nicotinic acid, 4 g KH_2PO_4, 4 g K_2HPO_4, 1 g NaCl, and 0.2 g $MgSO_4.7H_2O$ per liter of water. Ten-ml amounts in tubes were inoculated heavily and incubated with agitation at 28°C. The two supposedly nitrate-negative isolates of *E. cypripedii* and two typical isolates grew equally well. Moreover, after 24 hours' growth in the synthetic nitrate medium, nitrite accumulated in amounts detectable with the usual reagents, sulfanilic acid and alpha-naphthylamine. Growth was complete after 48 hours; neither nitrite nor nitrate could be detected. It is clear that *E. cypripedii* is capable of nitrate reduction under conditions in which this reaction is necessary for growth.

Other species of *Erwinia*, e.g. *E. amylovora*, that yield negative tests for nitrite in the usual nitrate-peptone medium, also grew and produced nitrite in the synthetic nitrate medium.

Discussion and conclusions.—*Identity of the pathogen with Bacillus cypripedii Hori.*—The major basis for our belief that the organism described here

TABLE 1.—Biochemical activities of some Erwinia species

Characteristic	Pectobacteria	*E. cypripedii*	*E. nigrifluens*	*E. amylovora*
Pectate gel liquefaction	+[b]	−	−	−
Galacturonate fermentation	+	+	−	−
Glucuronate fermentation	+ or −[b]	+	+	−
Mucate fermentation	+	+	+	−
Pentose fermentation (xylose, arabinose, rhamnose)	+ (arab. −)	+	+	−
Lactose fermentation	+ or x	x	−	−
Maltose fermentation	− (x) (+)	+	−	−
Cellobiose fermentation	+	+	+	−
Gas from sugar fermentations	+ or −	+ (−)	−	−
Acetylmethylcarbinol (V-P test)	+ or −	−	x	x
Citrate utilization	+	+	−	+
Tartrate, malonate utilization	+ or −	+[c]	−	−
Gelatin liquefaction	+	x	x	+ or x
Nitrate reduction[a]	+	+ (−)	−	−

[a] Formation of nitrate in nitrate-peptone broth.

[b] Reactions are recorded by the following conventions: + or −, approximately equal numbers of isolates are consistently positive or consistently negative; ( ), the reaction indicated is displayed by only a few isolates; x, a delayed positive reaction.

[c] Tartrate is fermented, yielding acid and gas.

is identical with the brown rot pathogen named by Hori is the exact correspondence of the disease symptoms seen in California with the symptoms described in Japan (8).

Methods for characterization of bacterial cultures have changed considerably in the past half century. Of the characteristics by which we distinguish *E. cypripedii* from *E. carotovora*, only one, action on gelatin, appears in Hori's communication. Although Hori's synopsis states that *Bacillus cypripedii* liquefied gelatin rapidly, his detailed description clearly indicates a delay of several weeks before significant liquefaction began. Delayed liquefaction of gelatin was characteristic of *E. cypripedii* as described in the present report. With one exception, there is no disagreement between the characteristics of the organism described here and the brown rot pathogen studied by Hori. The exception is in the Gram reaction: Hori considered *Bacillus cypripedii* Gram-positive. Since *Bacillus carotovorus* was also first described as Gram-positive (9), interpretation of the Gram reaction has evidently changed.

Taxonomic position of Erwinia cypripedii.—Division of the genus *Erwinia* into the pectolytic group and the non-pectolytic group of pathogens has been recognized for some time. The former group, *E. carotovora* and related soft rot organisms, has been proposed as a separate genus *Pectobacterium* by Waldee (14). The non-pectolytic group, which includes the type species *E. amylovora*, is sometimes referred to as the "true *Erwinia*" group. The decision as to which group (or genus) *E. cypripedii* most appropriately belongs is not an easy one, for in many ways this organism seems to show affinities intermediate to the pectobacteria and *E. amylovora*, as shown in Table 1.

1) *Comparison with the pectolytic group.*—*E. cypripedii* lacks the characteristic by which the pectobacteria can be so clearly circumscribed, i.e., enzymatic hydrolysis of pectate gels. There are also certain positive attributes of *E. cypripedii* that distinguish this pathogen from the pectobacteria. Fermentation of tartrate to yield acid and gas is a characteristic of *E. cypripedii* that is unique in *Erwinia*. Tyrosinase activity, as shown in the synthetic tyrosine medium, is another distinctive attribute of *E. cypripedii*. Maltose is readily attacked by *E. cypripedii*. In contrast, few isolates of the pectobacteria ferment this sugar; only one isolate studied (13) did so promptly.

2) *Comparison with the "true Erwinia" group.*—The lack of pectolytic activity and the inability of two isolates to form nitrite in the usual test medium suggest a relationship of *E. cypripedii* to the "true *Erwinia*" group. But since *E. cypripedii* attacks many test substrates that are not metabolized by the type species, *E. amylovora*, the two organisms cannot be closely related. There are, however, other pathogens whose characteristics suggest taxonomic positions intermediate to the above two organisms.

Table 1 shows that *E. cypripedii* is metabolically the most versatile of the non-pectolytic species of *Erwinia*. The table is arranged to show the gradation of metabolic reactivity, i.e., the decreasing number of substrates metabolized by *E. cypripedii*, *E. nigrifluens* (15), and *E. amylovora*, in that order. Of the other "true *Erwinia*" species, *E. salicis* attacks only 2 of the substrates shown in the table— maltose and cellobiose. The organism does, however, form gas from the sugars fermented. *E. tracheiphila* metabolizes few substrates, none of which appear in the table.

Since *E. nigrifluens* has become known only recently it is quite possible that additional non-pectolytic fermentative phytopathogenic bacteria will be found. Future study of such organisms would help clarify the taxonomic relationships of the non-pectolytic species of *Erwinia*.

Taxonomic conclusions.—Interpretation of the present evidence, pathological and bacteriological, leads to the following conclusions:

1) *E. cypripedii* should be again recognized as a distinct species of phytopathogenic bacterium.

2) *E. cypripedii* cannot be included in the soft rot group of *Erwinia*, nor in the genus *Pectobacterium*

Waldee.

3) *E. cypripedii* shows taxonomic affinities to the "true *Erwinia*" group of phytopathogenic bacteria.—Department of Bacteriology, University of California, Davis, and Department of Plant Pathology, University of California, Berkeley.

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EFFECT OF PLANT BED TEMPERATURE AND SEED POTATO DIP TREATMENTS ON INCIDENCE OF SWEET POTATO SPROUT DECAY CAUSED BY DIAPORTHE BATATATIS

Robert H. Daines, Eileen Brennan, and Ida A. Leone

Respectively, Plant Pathologist, New Jersey Agricultural Experiment Station, New Brunswick, and Assistant Research Specialists, New Jersey Agricultural Experiment Station, New Brunswick.

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SUMMARY

Plant bed temperature was found to influence the amount of dry rot (*Diaporthe batatatis*) on sweet potato sprouts. Disease incidence was greater at 85° than 75° or 90°F. Mercurials were the most effective seed-potato dips, with Tersan OM, Dyrene, and captan providing reduced protection in the order listed.

Introduction.—Dry rot (*Diaporthe batatatis* Harter & Field) of sweet potatoes, first described by Halsted (4), in 1890, is today recognized as one of the most destructive diseases affecting sweet potatoes during the storage period. Smoot and Jeffers (8) reported *D. batatatis* as one of the two fungi most frequently isolated from Maryland Golden sweet potatoes affected with end rot. This disease is frequently the most common disease of sweet potatoes in storage houses in New Jersey. It has also been reported in North Carolina, Texas, Virginia, Alabama, and Indiana (6: p. 68), Mississippi (3), and Arkansas (7) as a serious storage problem. Less is known about its importance as a plant bed or field disease. However, Harter and Field (5: p. 37) said: "The disease also occurs in the hotbed and in the field. The organism is not a vigorous parasite and consequently under ordinary conditions does not become evident until some time after harvesting. Diseased vines have been collected in abandoned hotbeds." From the evidence presented here, dry rot apparently occurs much more commonly in plant beds than the Harter and Field statement implies. Dry rot sprout decay can easily be mistaken for black rot. In its active form the disease imparts a black to dark-reddish-brown color to the base of the sprouts, and fruiting bodies are not observed at this stage. In more advanced stages, where sprouts are killed, they develop, on drying, a reddish-brown color and may show numerous small erumpent pycnidia in which are found many small hyaline one-celled spores. The conidia are fusiform to elliptical, predominantly two-guttulate, and usually measure 6–8×3–5 μ.

Review of literature.—In tests of temperature relation to pathogenicity of *D. batatatis*, Smoot and Jeffers (8) found that the percentage of infected sweet potatoes was about the same at 50, 60, 70, and 80°F, but that rot increased with increased temperature. Harter and Field (5), working with the fungus in Petri dishes, reported growth over a temperature range of 42–89°F, with best growth at 73–87°F.

Despite annual losses from this disease, the authors found no suggestions on control in the literature. This study presents information on the effects of chemicals and plant bed temperature in controlling the disease.

Experimental methods.—The methods used are the same as outlined in a previous paper (1). In this investigation sweet potatoes inoculated with spores of *D. batatatis* were held 24 hours at 85°F and a minimum relative humidity of 90%.

TABLE 1.—The effect of sweet potato dip treatments and plant bed temperatures on the development of dry rot in the resulting sprouts [a]

Fungicide treatments (lb./gal.)	Sprouts showing dry rot						Disease (av. 3 temperatures)	
	75°F		85°F		90°F			
	angle [b]	%	angle	%	angle	%	angle	%
Untreated check	53.6	64.8	64.4	81.3	44.8	49.6	54.3	65.9
Semesan Bel (1/7½)	10.4	3.3	15.3	7.0	7.3	1.6	11.0	3.6
Coromerc (1 pt./10)	5.9	1.1	19.3	10.9	6.7	1.4	10.6	3.4
Tersan OM (1/5)	28.1	22.2	34.8	32.6	27.5	21.3	30.1	25.2
Dyrene (1/5)	32.5	28.9	37.9	37.7	26.1	19.4	32.2	28.4
Captan (1/5)	35.7	34.1	45.8	51.4	35.7	34.0	39.1	39.7
Average %		21.6		35.0		17.4		24.3
Angle	27.7		36.3		24.7		29.6	
LSD 5%	3.5		3.5		3.5		2.0	
LSD 1%	4.7		4.7		4.7		2.7	

[a] 1 year's data.

[b] angle = arcsin $\sqrt{\text{per cent.}}$

Results and discussion.—The treatments (Table 1) were used at three temperature levels: 75, 85, and 90°F. The data (Table 1) indicate that, for each fungicide used, disease incidence was highest in the 85° bed. A single year's data cannot be analyzed statistically, but it seems safe to conclude that the trend obtained was significant. Maintaining a selected bed temperature apparently would help control dry rot, but such help would not be as meaningful as it is for black rot (1) or Java black rot (2).

The effect of plant bed temperature on this disease agrees well with laboratory studies on the effect of temperature on mycelial growth of *D. batatatis* in agar plates (Fig. 1.)

All seed potato dip treatments used provided highly significant control over the untreated check at each temperature level. Semesan Bel (2-chloro-4-(hydroxymercuri) phenyl and isomers, 3.2%), 4-(hydroxymercuri)-2-nitrophenyl and isomers, 12%), and Coromerc (*n*-phenylmercuriethylenediamine, 10%) provided the most effective control of all treatments. At each temperature they provided highly significant control over all other treatments. Tersan OM (tetramethylthiuramdisulfide, 45%, and hydroxymercurichlorophenol, 10%), Dyrene (2,4-dichloro-6-(*O*-chloroanilino) triazine), and captan (*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide) provided control of sprout decay in the order listed, with captan least effective.

From the data presented it is clear that the incidence of dry rot on sprouts in the plant bed is influenced by the temperature of the plant bed and the fungicide dip treatment employed.—New Jersey Agricultural Experiment Station, New Brunswick.

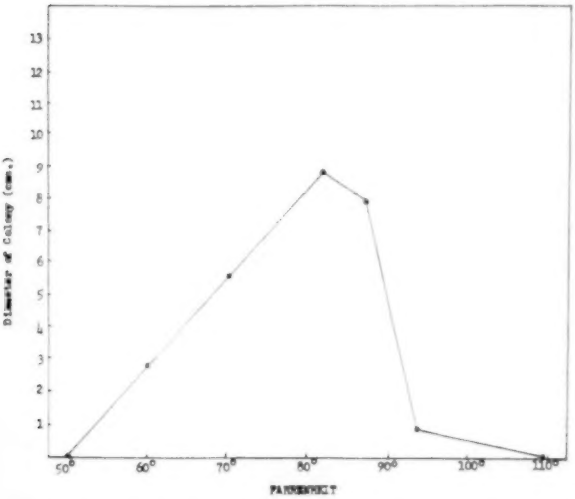

Fig. 1. Effect of temperature on the mycelial growth of *Diaporthe batatatis*. Five-day-old agar cultures.

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FLOWERING CHERRY, A RESERVOIR OF THE LITTLE CHERRY VIRUS

J. M. Wilks and E. L. Reeves

Respectively, Plant Pathologist, Canada Department of Agriculture, Summerland, British Columbia; Pathologist, Crops Research Division, ARS, U.S.D.A., Wenatchee, Washington.

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SUMMARY

Comparisons of the K&S disease and the little cherry disease at Creston, British Columbia, and additional tests of the K&S disease at Wenatchee, Washington, have shown no differences between them. The fruit and foliage symptoms are identical on all sweet cherry varieties tested, and rates of spread are comparable through inoculated trees. Foliage symptom expression is more severe at Wenatchee than at Creston. These findings, combined with those from earlier studies, justify considering these viruses the same, and applying the name "little cherry" to the virus in flowering cherry. *Prunus serrulata* trees that were growing in the Kootenay region of British Columbia when the little cherry disease appeared there have been shown to carry little cherry virus.

Reeves et al (7) reported that a latent and widely occurring virus in oriental flowering cherry varieties causes symptoms in sweet cherry similar to those of the little cherry disease (1, 2). The identity of the two diseases thus needs further investigation.

A number of virus diseases of sweet cherry in western North America resemble one another in having as their most distinctive symptom a failure of the fruits to develop normal size and color. These diseases are buckskin, little cherry, small bitter cherry, western X little cherry, and albino (8). The widely separated areas of natural occurrence of these diseases, and the danger of transferring them beyond the limits of their natural occurrence, restrict opportunities for comparing them directly in the same locality. Nevertheless, reasons have been given for considering little cherry and small bitter cherry distinct (3), and it has been shown that little cherry and western X little cherry have different effects in some hosts (8). On the other hand, evidence indicates that western X little cherry and small bitter cherry are synonymous (4). Buckskin and little cherry differ in host range and rate of spread in common hosts (2), and albino differs from little cherry in its syndrome in sweet cherry (10).

Little cherry was first reported (1) from an orchard in the West Kootenay area of British Columbia, in 1933. Spread of the disease was very rapid in individual trees and from tree to tree. Within 15 years the disease had spread to districts as much as 50 miles distant and caused practically 100% infection of the cherry trees within that locus. Trees characteristically become completely infected within 2 years of the inoculation of a single branch (8). The symptoms of the disease in most commercial varieties of sweet cherry are restricted to a reduction in the size and sugar content of the fruit at harvest time. Fruits borne on diseased trees are not just delayed in maturity, but their development is arrested and they never reach the maturity of fruits on normal trees. Certain sweet cherry varieties and seedlings produce early autumnal red leaf symptoms (9). Diseased sweet cherry trees have comparable fruit symptoms whether established on mazzard or mahaleb rootstocks. Peach and chokecherry are immune to the little cherry virus (8).

A latent virus in flowering cherry was reported first in 1955, in the *Prunus serrulata* Lindl. varieties Kwanzan and Shiro-fugen (7). Four additional varieties of flowering cherries (Amanogawa, Fugenzo, Taki-nioi, and Autumnalis) were subsequently found carrying the virus naturally. Diseased trees of these varieties were normal in appearance and growth (6). Naming the virus found in flowering cherries was postponed until comparisons could be made with other viruses that cause small cherry symptoms. The virus was temporarily called "K&S," for the first letters of Kwanzan and Shiro-fugen. In 1958 it was reported to differ from western X little cherry virus in the symptoms it induced in sweet cherry, chokecherry, and peach (6).

Comparison showed that the fruit symptoms caused by the K&S virus on infected sweet cherries in Washington were identical with those caused by the little cherry virus in British Columbia (7). However, there was some doubt that the foliage symptoms in sweet cherry were identical. The K&S virus moved through individual trees at a rate comparable with that of the little cherry virus. The identity of the two viruses was further strengthened by similarity of fruit symptoms in sweet cherry trees established on mazzard or mahaleb rootstocks, by lack of symptoms in peach and chokecherry, and by non-recovery of the virus from inoculated peach and chokecherry trees (6).

This paper compares symptoms caused by the two viruses on common test hosts in the Kootenay area, British Columbia, and describes symptoms of the K&S virus in the same test hosts at Wenatchee, Washington. It also assesses accumulated data now available for comparing the symptoms caused by the K&S and little cherry viruses.

Materials and methods.—*British Columbia.*—Work was conducted at Creston, within the area of natural occurrence of little cherry.

All little cherry inoculum was obtained from Lambert cherry trees. The K&S inoculum was obtained from a Kwanzan clone at Summerland, British Columbia, a district free of little cherry. All inoculations were made by T-budding with a minimum of 5 buds per test tree.

Two test hosts particularly used for comparing the 2 diseases were Van and Bing cherries. Uniform trees of the same clone on rootstocks from the same seed source were used.

The tests of Van cherry included 2 trees inoculated

with K&S virus only; 2 trees inoculated with little cherry virus only; and 6 trees inoculated with both viruses, 3 with the K&S-infected buds inserted above the little cherry-infected buds and 3 in a converse arrangement.

Eleven bearing Bing cherry trees were inoculated with K&S virus, and 2 were inoculated with little cherry virus. These tests merely augmented earlier little cherry investigations, in which larger numbers of Bing trees were inoculated.

Three healthy bearing Lambert cherry trees were inoculated with the K&S virus for comparison with trees of this variety previously inoculated with little cherry virus.

Foliage symptoms of K&S disease on 1- and 2-year-old trees of the sweet cherry varieties Sam and Star maintained in screen cages were previously obtained during the indexing of flowering cherry trees growing in the Kootenays.

Washington.—Work was conducted at Wenatchee, outside the area of natural occurrence of little cherry. Although symptoms caused by the so-called K&S and little cherry viruses could not be directly compared in Washington on trees growing under the same environmental conditions, results were correlated with those at Creston, by employing the same clones of some test plants and making inoculations at comparable stages of plant growth.

One source of the K&S inoculum used at both locations was a Kwanzan clone at Summerland, B. C. Other flowering cherries were also used as sources of inoculum at Wenatchee, including the Kwanzan and Shiro-fugen clones reported on in previous work (5). All inoculations were made by T-budding, with a minimum of 3 buds per test tree.

Ten of the Bing and 10 Van test trees used at Wenatchee were from clones of the same variety, propagated on the same rootstock source, that were used in tests conducted at Creston. Four 2-year-old and two 6-year-old Lambert trees were also used. These tests augmented larger numbers of fruiting Bing, Van, and Lambert trees previously infected in other investigations of the K&S virus in the same experimental plots.

Foliage symptoms of the K&S disease on the varieties Sam and Star were obtained by placing buds of these varieties high on branches of previously infected Bing trees and forcing them into growth for 2 years.

Results.—*Van cherry.*—At Creston, no fruits were produced on the test trees in the year following inoculation. However, foliage symptoms were present by midsummer in all trees inoculated with either of the viruses and on trees inoculated with both viruses, but were absent in uninoculated check trees. The symptoms included upcurling of leaf margins, interveinal reddening, and eventual dropping of basal leaves on current-year terminal growth. The foliage reddening became apparent by the beginning of August and intensified until the end of September. At this time, normal autumn coloration was beginning to appear in the foliage of uninoculated trees.

In the second season, foliage and fruit symptoms were apparent on all inoculated trees. The foliage symptoms in both seasons and the fruit symptoms in the second season were identical on all inoculated trees regardless of the source of inoculum.

At Wenatchee, the fruit and foliage symptoms on Van were similar to those on this variety at Creston.

Bing cherry.—During the 2 years in which observations were made at Creston the foliage symptoms resembled those described for Van cherry, but were less pronounced. Fruit and foliage symptoms were identical on trees inoculated with either the K&S or the little cherry virus. Uninoculated trees produced normal fruits and leaves without curling or reddening.

At Wenatchee, the fruit and foliage symptoms in Bing were comparable.

Star and Sam cherries.—At Creston the foliage symptoms on trees of these varieties inoculated with buds from the standard Kwanzan source and from flowering cherry sources found in the Kootenays were identical with the symptoms on trees infected with the little cherry virus.

At Wenatchee, these varieties expressed good foliage symptoms, similar to those observed at Creston, in 1957 and 1958 on growth from buds of these varieties high on branches of infected Bing trees in 1956.

Lambert cherry.—At Creston, no foliage symptoms appeared in Lambert trees inoculated with the K&S virus. They have never been observed in Lambert trees infected with little cherry virus. In the year following inoculation, fruits on the inoculated branches displayed symptoms identical to those of little cherry.

At Wenatchee, the fruit symptoms were similar to those on Lambert at Creston. In 1958 at Wenatchee, infected Lambert trees had only vague leaf symptoms just before the early autumnal foliage coloration appeared on normal trees in late October. However, during the 1957 season, when more distinctive foliage symptoms developed on all varieties, Lambert showed fair leaf symptoms in late September.

It was previously indicated (5) that weather influences the development of distinctive foliage symptoms on infected trees in some seasons. There are also indications that foliage symptoms may be slightly more pronounced at Wenatchee than at Creston. This may be due to a longer fall growing season that permits further development of foliage symptoms prior to early autumnal colorations.

Indexing of flowering cherry trees in the little cherry area.—A survey was made throughout the area of little cherry disease distribution in the Kootenays to determine the number and distribution of flowering cherry trees. Twenty-seven trees were found. Nineteen were identified as belonging to the species *Prunus serrulata*, but variety identifications were not made. All trees were indexed for K&S virus on young trees of Sam cherry growing in screen cages. All 19 trees in the species *P. serrulata* were infected, as evidenced by production of upward curling and premature reddening on the leaves of Sam cherry test trees. None of the flowering cherry trees of other

species indexed positive; these trees are being held for further observation.

Six of the above-mentioned flowering cherry trees, 3 identified as *P. serrulata*, are growing in the Willow Point district within 5 miles of the orchard in which little cherry was first found, in 1933. These trees had been planted before 1933. The 3 *P. serrulata* trees were found to be infected.

Discussion and conclusions.—Previous investigations of the K&S virus in Washington and of little cherry in British Columbia have indicated that the little cherry virus and the K&S virus gave the same fruit symptoms in several varieties of sweet cherry; that neither virus could infect peach or the native chokecherry; that both produced fruit symptoms equally in sweet cherry trees on mazzard or mahaleb rootstock; that the rate of distribution of both was rapid in test sweet cherry trees; and that both were transmitted readily by budding. However, foliage symptoms appeared to be more severe following inoculation of Van, Bing, and Lambert varieties with K&S virus at Wenatchee.

The tests described in this paper have shown that the foliage symptoms were identical when the viruses were introduced simultaneously into separate comparable trees of these varieties growing in the same location, indicating that differences noted hitherto should be ascribed to climate or soil rather than to differences in symptom expression. These tests have also confirmed that the spread of the two viruses through inoculated trees, as indicated by symptom expression, is the same. Therefore, there seems adequate justification for considering that the viruses are synonymous, and for substituting the name "little cherry" for the temporary name "K&S."

The discoveries that flowering cherry trees were growing in the Kootenays close to the location of the first outbreak of little cherry when it occurred, and that these trees now carry little cherry virus suggest the interesting possibility that they were the source of infection of sweet cherry trees in that area. However, it is now impossible to know whether these trees carried the virus when they were introduced to the area or whether they were since infected naturally from diseased sweet cherry plantings.

The apparent identity of these two viruses, the status of little cherry as one of the most serious of the known diseases of sweet cherry, and the widespread occurrence of the virus in flowering cherry trees suggest that until virus-free clones of flowering cherry are available, precautions should be taken to prevent plantings of flowering cherries near sweet cherry plantings in areas where little cherry does not occur.

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CERCOSPORA BLIGHT OF SNAPDRAGON

Curtis R. Jackson

Assistant Plant Pathologist, Gulf Coast Experiment Station, University of Florida, Bradenton.

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SUMMARY

Infection of snapdragon leaves and stems by *Cercospora antirrhini* results in stunting and defoliation of plants. The incubation period was 21–30 days, and mycelial growth *in vitro* was maxmum at 24–28°C. Mean lengths of conidia and conidiophores exceeded the upper limits of reported length ranges.

A blight of snapdragon, *Antirrhinum majus* L., caused by *Cercospora antirrhini* Muller & Chupp was recently observed to cause extensive defoliation of plants in gardens and commercial nurseries in Florida. The disease was first reported in 1950, from Guatemala by Muller and Chupp (4); in 1959 Bolick (1) reported it from Florida, the first record in the United States. This is a report on disease symptoms and data concerning the morphology and physiology of the pathogen.

Leaf symptoms.—Symptoms on naturally infected and greenhouse-inoculated plants are similar to the descriptions given by Chupp (2) and Muller and Chupp (4). Lesions are subcircular, 0.5–7 mm in diameter, discrete to confluent (Fig. 1), with dull-white or gray centers surrounded by light-brown, slightly raised margins. The central portions of the lesions are ½–5/6 of the total diameters. Under very humid conditions, lesions may expand rapidly, leading to the development of irregular, poorly defined, gray or tan necrotic areas that extend to 15 mm in diameter and occasion-

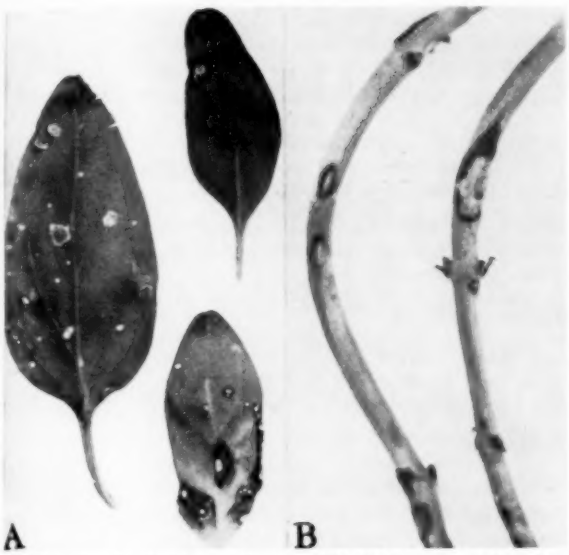

Fig. 1. Leaf (A) and stem (B) lesions 8 weeks after inoculation.

ally exhibit faint concentric zonation. Sporulation may be abundant on the central portions of lesions, and heavily infected leaves become chlorotic and eventually drop off.

Stem symptoms.—Stem lesions, which occur predominantly on the basal portions near the soil, are depressed, dull white to gray, with brown margins, at first discrete, subcircular, oval, or elliptical, 1–10 × 1–5 mm, becoming confluent and up to 4 cm long (Fig. 1). The cortical tissue of ¼–¾ of the stem circumference becomes necrotic and develops minute longitudinal cracks. Sporulation is sparse on lesion surfaces. Plants with stem and leaf infections are stunted and chlorotic.

Inoculation.—Isolations from stem and leaf lesions consistently yielded the pathogen. Growth of the fungus was entirely mycelial on V-8 juice (Campbell Soup Co.) and potato-dextrose agars (PDA) when cultures were started from single transfers of mycelium. Sporulation was sparse when PDA plates were flooded with a suspension of mycelial fragments. A suspension of conidia and mycelial fragments from sporulating cultures was atomized on 8-week-old snapdragon plants growing in flats in the greenhouse. After 3 days in a moisture chamber, half of the plants were removed to a greenhouse bench and half were planted in the field. Initial leaf and stem symptoms appeared on all inoculated plants within 21–30 days. Temperature range during the incubation period was 60–90°F. The pathogen, when reisolated from stem and leaf lesions, was essentially similar to the original isolates.

Causal organism.—The morphology of the pathogen from leaf lesions of field- and greenhouse-grown plants (Fig. 2) conformed in most respects to the original description by Muller and Chupp (4). However, numbers of conidiophore septa were generally higher and dimensions of conidia and conidiophores were more variable. Most conidia had bluntly rounded bases. The measurements of 50 conidia (standard error given) were 32–420 μ (mean 145.5±11.8 μ) long, 1.3–6.4 μ (mean 3.2±0.2 μ) wide at midpoint, and with 2–29 (mean 10) septa. Twenty-five conidiophores were 51–299 μ (mean 129.2±10.3 μ) long, 2.8–7.6 μ (mean 4.1±0.3 μ) wide, and with 1–16 (mean 4) septa.

The large variations in the sizes of both structures reflect the extreme differences found in small random samples of conidia and conidiophores in various stages of development. Mean lengths of conidia and conidiophores exceeded the upper limits of previously reported length ranges, and length and width ranges were greater than have been reported.

Cultural characteristics.—The fungus was cultured on V-8 juice-mineral liquid medium composed of 180 ml V-8 juice, 1 g ammonium nitrate, and 1 g dibasic potassium phosphate per liter of medium. One-hundred-ml portions of the medium were placed in 500-ml Erlenmeyer flasks and inoculated with 3-mm discs of a PDA culture of the fungus. Six flasks were selected at random and placed in each of 5 chambers at 16, 20, 24, 28, and 32°C. About 32 ft-c of constant

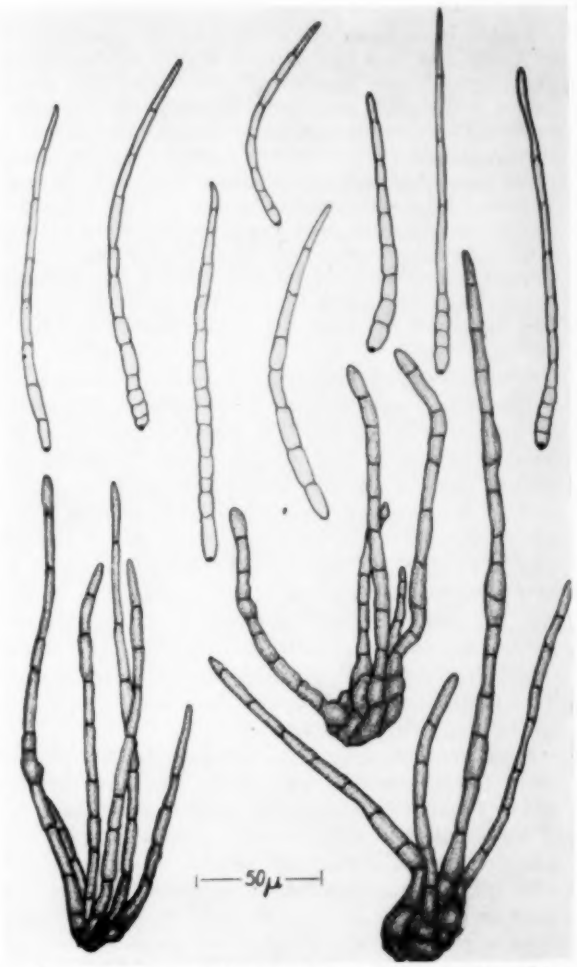

Fig. 2. Camera lucida drawings of conidia and conidiophores of *C. antirrhini* from host.

tungsten illumination was provided in each chamber during the 21-day undisturbed growth period. The contents of each flask were collected on previously dried and weighed filter papers. Dry weights of mycelia were obtained after drying at 32°C for 4 days. The data were analyzed with Duncan's (3) multiple range test.

Mycelial growth was entirely submerged below the medium's surface at unfavorable temperatures, but scattered emergent mats developed at 24 and 28°C. Growth was maximum at 28°C. but, at the 5% level of significance, not greater than growth at 24°C. The weight of mycelial growth at 24 and 28°C was significantly different from that at 20, 32, and 16°C. No differences in growth were found among the latter 3 temperatures. — Gulf Coast Experiment Station, University of Florida, Bradenton.

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VARIATIONS IN RESPIRATORY RESPONSES IN FUSARIUM-INFECTED TOMATO PLANTS

R. P. Scheffer

Associate Professor, Michigan State University.

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SUMMARY

Leaves from Fusarium-infected tomato plants grown in a low-N regime had a much higher oxygen uptake than leaves from uninfected low-N controls. Plants grown in a high-N regime did not respond to infection by increased oxygen uptake by leaves. The degree of stimulation of oxygen uptake varied with the season, being maximum under conditions of high light intensity and long days, and minimum under low-light short-day conditions. Results were comparable for stem tissue containing the causal fungus in minute amounts. Since disease can develop on schedule with no quantitative change in oxygen uptake by host tissues, results suggest that such disturbances may be secondary factors in pathogenesis.

Infection of tomato plants by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans. increases carbon dioxide production and oxygen uptake by leaves that do not contain the fungus, before visible symptoms occur (4). Similar changes have been observed in leaves of several species locally infected by rust and mildew fungi (2, 9) and in sweet potato tissue infected by *Ceratostomella fimbriata* (Ell. & Halst.) J. A. Elliott (1). Increased respiration is often regarded as a general response of plant tissue to disease. The present report describes further work on quantitative changes in respiration of Fusarium-infected tomato plants, with special emphasis on variability.

For several reasons, both leaf and stem tissues were used. Leaf blades do not contain the causal fungus, and any change in metabolism might suggest the action of toxins carried with the transpiration stream from infection sites. For example, lycomarasmin and fusaric acid inhibit succinoxidase and cytochrome oxidase activities (7). If these toxins are important factors in pathogenesis, their effects should be detectable, and a study of respiratory changes in diseased leaves should help to clarify the question of toxins in the wilt diseases. In stem tissue the pathogen is confined to the xylem tubes, which make up a small part of the total volume of the stem. The contribution of the fungus should therefore be minute in relation to gas exchange by host tissue, and stem tissue should be useful for studies of pathogenesis.

An attempt was made to determine the conditions necessary for maximum and predictable respiratory stimulation by infection. Plants were grown before and after inoculation under various nutritional and temperature conditions, and in different seasons. Such information is necessary for more detailed study of "pathological respiration." The data reported here show variations in response associated with certain conditions. Surprisingly, under some conditions there was no stimulation of oxygen uptake by infection.

Materials and methods.—Tomato plants of the variety "Bonny Best" were grown in the greenhouse in peat-sand mixtures and were watered with a suitable concentration of a soluble fertilizer. Plants were inoculated 21–35 days after seeding, and were usually held at 22°C night temperature after inoculation. Daytime temperature was not strictly controlled in the 22° house, but was always at least 6°C higher than night temperature. During spring, summer, and fall, the daytime temperature often exceeded 30°C. The 20° and 28° houses were maintained at those levels both night and day. Otherwise, the methods of growing plants and inoculum and of inoculation were as previously described (4).

Gallegly and Walker's solutions (5) were used to grow plants under different nutritional conditions. The basal (1H) solution was reduced to one-tenth (0.1H) and to one-half (0.5H), and was doubled (2H) and tripled (3H). High-nitrogen, high-phosphorus, and high-potassium solutions were prepared by tripling the amounts of the respective ions contained in the basal solution. Solutions for low nitrogen, phosphorus, and potassium were prepared by reducing the amounts of these ions contained in the basal solution to one-tenth of normal. All other constituents were kept at the 1H level. Osmotic pressures of the solutions were usually kept at the same level by adding calculated amounts

of sodium chloride. In one experiment, sodium chloride was omitted from half the plants to be sure that results were not associated with the level of sodium chloride. All solutions contained the same amounts of microelements (A-Z solution) (5).

Seedlings for the nutritional experiments were started in clean sand and set in 8-in. varnished clay pots containing pure quartz sand (grade no. 4 crushed stone). An automatic siphon maintained equal water level in each pot (5), and pots were flushed daily with nutrient solution. Five plants were grown in each pot for about 30 days before inoculation. Replications were used.

Oxygen uptake was determined with a Warburg apparatus. Leaves were carefully selected for uniformity, using the 3rd or 4th leaf above the cotyledons, and disks were cut from the blades with an 8-mm cork borer. In all cases, leaves were used before wilt symptoms occurred, but in most cases the leaves in late stages of disease were yellowing. Each experiment was run with duplicate or triplicate flasks for each treatment, and each flask contained equal numbers (20 or 30) of leaf disks. In all experiments not requiring infiltration of fluids, leaf disks were placed in flasks on wet filter paper (4). Flasks were equilibrated for 30 minutes in the dark, and oxygen uptake data were taken for 2 hours at 30°C in the dark. Data are reported as oxygen uptake per 10 mg dry wt. In previous work, oxygen uptake per unit dry wt. was found to agree well with oxygen uptake per unit tissue nitrogen (4). Addition of substrates usually did not increase oxygen uptake.

Respiration of stems was measured by the tissue slice technique previously described (4). Slices were placed in Warburg flasks on moist filter paper, or were placed in phosphate buffer solutions. Oxygen uptake by slices on moist paper did not differ significantly from that of slices in buffer solutions.

Special care was taken to ensure uniformity of tissue from flask to flask and from treatment to treatment. Equal numbers of disks or slices from 10 or more plants were randomized by thorough mixing before being placed in the Warburg vessels. In other experiments each flask contained the same number of disks from each of 10 or more leaves, taken from comparable parts of each leaf. Similar procedures were applied to stem slices. Such care kept the average variation between replicates to 2% in the experiments described, with a maximum of 8%. Respiration of healthy controls did not vary greatly from day to day in any one set of plants (Tables 1 and 2), but there was variation from one set of plants to another (4), presumably depending on growing conditions.

Results.—*Variation in disease-induced oxygen uptake by leaves*—Previous work (4) showed that both stems and leaves had increased oxygen uptake as a result of infection. However, the amount of stimulation reported varied from one set of plants to another. Attempts were made to find the factors responsible for the variations.

The respiratory responses of plants grown and maintained at 28°C were compared with the responses of plants grown at a minimum temperature of 20°C. Respiratory response to infection was most pronounced at the higher temperature, where disease developed most rapidly. A stimulated respiration was apparent in the lower-temperature plants, but it occurred later and was less pronounced.

Plants were grown in several levels of balanced Hoagland solution—0.1, 0.5, 1, 2, and 3H (5)—and inoculated, and oxygen uptake of inoculated plants and uninoculated controls was determined. The 0.1H plants, which developed symptoms most rapidly, had an infection-stimulated oxygen uptake (+63%) significantly higher than that of the other plants. There were no differences in maximum per cent stimulation of respiration between plants at the other nutrient levels (av., +22%), although all inoculated plants had significantly higher oxygen uptake than did healthy controls.

The respiratory responses of inoculated and uninoculated plants grown in high and low levels of N, K, and P, and in 1H balanced solutions, were compared. Leaf disks were placed in Warburg flasks on moist filter paper. The inoculated plants grown in high and low levels of P and K and in 1H solutions all had higher oxygen uptake than did uninoculated controls, but there were no significant differences in the levels of stimulation (av., +22%). Inoculated low-N plants had a great increase in oxygen uptake, as compared to

TABLE 1.—Effect of nitrogen level in the growing medium on respiratory responses of leaves to Fusarium infection

Days after inoculation	Tissue	High N [a]		1 H [a]		Low N [a]	
		O_2 uptake [b] (μl)	% increase	O_2 uptake (μl)	% increase	O_2 uptake (μl)	% increase
7	Healthy	39.7		34.2		34.6	
	Diseased [c]	39.7	0	42.3	24	40.5	17
9	Healthy	36.6		35.0		36.8	
	Diseased	36.4	0	39.9	14	56.6	54
11	Healthy	37.2		37.4		31.9	
	Diseased	41.5	12	46.8	25	53.9	69
14	Healthy	30.9		34.2			
	Diseased	30.9	0	38.3	12		

[a] High-N medium had 3 times the standard (1H) level, and low N medium had 1/10 the standard (1H) level of N. All other ions were held at the 1H level.
[b] O_2 uptake per 10 mg dry wt. per hour. Values are the averages of 2 flasks each.
[c] Symptoms at 7, 9, 11, and 14 days were respectively none, slight, moderate, and severe.

TABLE 2.—Influence of condition of plants on respiratory response of stems to infection

Days after inoculation	Tissue	July ("Hard") plants		Nov. (High-N) plants	
		O_2 uptake[a] (μ l)	% change[b]	O_2 uptake[a] (μ l)	% change[b]
1	Healthy	35.5		47.6	
	Diseased	27.5	−22[c]	47.5	0
2	Healthy	34.2		42.3	
	Diseased	46.1	+35	45.1	+7
4	Healthy	33.9		41.3	
	Diseased	50.7	+47	39.1	−5
6	Healthy			39.7	
	Diseased			37.3	−6

[a] O_2 uptake per 10 mg dry wt. per hour. Values are averages for 3 or 4 flasks each.

[b] Values are per cent increase or decrease in respiration after inoculation, compared with uninoculated controls.

[c] An early decrease, followed by an increase, was observed in several experiments.

uninoculated low-N plants. Inoculated and uninoculated high-N plants did not differ in oxygen uptake (Table 1). The inoculated low- and high-N plants developed symptoms at about the same rate under the conditions of the experiment, which was done in August, when plants had good light and long days.

The effects of high and low levels of nitrogen on respiratory responses to infection were compared in a second experiment, in June, when plants were grown under conditions of maximum light and longest days. In this experiment, leaf disks were placed in *M*/30 phosphate buffer, pH 5.7, in the Warburg flasks. Results were similar to those given in Table 1. The experiment was done a third time, in December, when light intensity in the greenhouse was minimum and days were shortest. In the third experiment, leaf disks were placed in Warburg flasks on moist filter paper. In this case, infected low-N plants had 17% higher oxygen uptake than low-N controls, whereas oxygen uptake by leaves from the high-N infected and uninfected plants was equal.

Variation in disease-induced oxygen uptake by stems—Plants were grown 30 days in high and low levels of nitrogen and inoculated by the root-dip method. The experiment was done in June, using greenhouse plants grown under the longest days at full light intensity. The high-N plants were dark green but lacked the soft, succulent appearance of high-N plants grown in other seasons. Possibly, the long days gave enough carbohydrate synthesis that C/N ratios were higher than in plants used previously. The low-N plants also were still "harder" looking than low-N plants in previous experiments. Respiratory responses in the stems of diseased and healthy plants were compared with the stem slice technique. Each Warburg flask contained 20 slices of comparable weight in *M*/30 phosphate buffer, pH 5.7. Oxygen uptake by stems from low-N infected plants was 17, 83, and 185% above oxygen uptake by uninoculated controls at (respectively) 9, 13, and 16 days after inoculation time. At the same times, stems from high-N infected plants had 3, 46, and 75% stimulation of oxygen uptake.

Respiratory responses in stems were compared further with a cutting inoculation technique (8). Cut stems were allowed to take up spore suspensions, and stem slices were cut from the second internode above the cotyledons. Oxygen uptake was again measured by the use of stem slices on moist filter paper. When this experiment was done in July, using stocky, "hard" plants, infection decreased oxygen uptake the first day, followed by significant increases (Table 2). The early decrease followed by an increase in respiration has been observed in other experiments. Other cuttings from this same group of plants, rooted after inoculation, developed mild disease symptoms. When the experiment was done in October, using "soft," succulent plants, infection caused no change in respiration for 9 days after inoculation. Again, in November, using high-N plants, infection resulted in no change in oxygen uptake, as compared to uninoculated cuttings (Table 2). Disease symptoms were severe in plants of the October and November experiments. Stems inoculated in this way are saturated with the fungus in the xylem vessels, in contrast to leaf-blade tissue, which does not contain the fungus (4).

Evidently, disease can develop on schedule with essentially no change in oxygen uptake by stem or leaf tissue (Tables 1, 2), or disease development can be accompanied by much augmented oxygen uptake, depending on plant condition and environmental factors.

Discussion.—There is much evidence that oxygen uptake and carbon dioxide evolution generally increase before symptoms develop in tomato plants with Fusarium wilt. This was shown first by Bloom (3), who measured respiration of whole leaves and whole tops of plants by gas trains and gravimetric analysis of carbon dioxide. In confirmation of Bloom's work, comparative gas exchange data on whole plants showed that disease generally increased respiration (unpublished). The present work and data of Collins and Scheffer (4) also support data by Naef-Roth (6) on the comparative respiration of diseased and healthy whole small leaves, as determined manometrically. Nevertheless, the increase in metabolic activity does not always accompany symptom development in leaves and stems. Plants grown in high-nitrogen regimes were shown to develop symptoms as expected without increases in oxygen uptake by leaf tissue. Stems also were shown to be capable of either increased oxygen uptake or of no significant change in oxygen uptake following infection, depending on the condition of the plant.

The results clearly show that the increase in oxygen uptake by leaves after infection is an incidental factor in Fusarium wilt pathogenesis. The data suggest that this may be true for the stems as well; at least, the possibility should be kept in mind that a study of "pathological respiration" may tell us very little about parasitism. However, several factors should be considered before this reasoning is carried too far regarding stems. Cells immediately in contact with the fungus

may be affected differently from the adjacent or more distant cells, and measurable reactions in cells away from the points of contact may completely obliterate the localized effects. Metabolism of the cells in direct contact with the pathogen may be of much more significance for an understanding of parasitism.

Disease must be traced ultimately to changes in metabolism, but the changes do not necessarily involve respiration directly. Even so, the experiments reported herein do not rule out the possibility of qualitative changes in respiration, such as the use of alternate pathways. However, no qualitative changes were definitely established for leaf tissue after hundreds of experiments with metabolic inhibitors and C^{14}-labeled substrates (unpublished). Similar experiments have not been done with stem tissue, which is more closely associated with the causal fungus. There is still no clear evidence for the action on leaf tissue of toxins carried in the transpiration stream.—Department of Botany and Plant Pathology, Michigan State University, East Lansing.

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MAGNESIUM NUTRITION OF NICOTIANA TABACUM IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS

Robert J. Shepherd and Glenn S. Pound

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SUMMARY

TMV-infected tobacco grown in liquid culture solutions containing various concentrations of magnesium developed typical deficiency symptoms and were retarded in growth to varying degrees, depending on the concentration of the element supplied. TMV concentrations were lower in magnesium-deficient plants, as shown by virus assays with expressed saps or extracts from dry tissue samples. Differences in the TMV concentrations of deficient and nondeficient plants were not great at any period after inoculation, but virus yields were consistently less from severely deficient plants.

Plant viruses are not known to possess any enzymatic activity, and thus depend on the host cell for energy required and precursors necessary to form the final infectious virus particles. At present the virus is envisaged as providing the host cell with a template for the replication of similar virus units while at the same time competing with other systems of the host protoplasm for the constituents that enter into the formation of virus. Both the virus and the living functional host cell are essential systems in this synthesis. Hence, a study of the nutritional requirements of the host in relation to the rate of multiplication of the virus may assist in elucidating the chain of events leading to the formation of complete virus.

Previous investigations have shown that virus concentration may vary widely in hosts grown under different conditions of mineral nutrition. This dependence of virus multiplication upon the nutritional status of the host has been described for a number of nutrient elements and various host-virus combinations.

Several of the microelements, in addition to the major mineral nutrients, have been shown to have marked effects on the multiplication of the tobacco mosaic virus (TMV) in tobacco. Zinc affects multiplication of TMV in a manner corresponding to its effect on host growth (2), whereas manganese has the reverse effect. Welkie and Pound (9) found TMV in the highest concentrations in plants severly deficient in manganese. Iron and boron deficiencies, however, had no marked effect on virus multiplication. Pound and Welkie (7) observed that TMV multiplication was limited by inadequate iron only in severely deficient plants. Shepherd and Pound (8) found that, following a short lag after inoculation, TMV increased to a concentration as great in boron-deficient plants as in nondeficient plants. The present study concerns the effects of magnesium nutrition on the multiplication of TMV in tobacco.

Materials and methods.—The methods used in culturing test plants and determining virus concentrations were those used previously (8).

TABLE 1.—*Chemical composition of nutrient solutions used to obtain different levels of magnesium*

Salt	Molarity of stock solution	Amount (ml) of stock solution per L of final solution to get indicated concentration of magnesium (ppm)				
		0.0	0.5	5	48	486
$CaCl_2 \cdot 2H_2O$	0.5	0.0	0.0	0.0	0.0	20.0
$Ca(NO_3)_2$	1.0	10.0	10.0	10.0	10.0	0.0
KCL	1.0	0.0	0.0	0.0	0.0	5.0
KH_2PO_4	0.2	5.0	5.0	5.0	5.0	5.0
KNO_3	2.0	5.0	5.0	5.0	5.0	5.0
K_2SO_4	0.2	5.0	5.0	5.0	5.0	0.0
$MgCl_2 \cdot 6H_2O$	0.1	0.0	0.2	2.0	20.0	40.0
$MgNO_3 \cdot 6H_2O$	1.5	0.0	0.0	0.0	0.0	10.0
$MgSO_4 \cdot 7H_2O$	0.1	0.0	0.0	0.0	0.0	10.0
NaCl	2.5	8.0	8.0	8.0	8.0	2.0

Nicotiana tabacum L. var. Havana 38 plants were grown in liquid nutrient solutions containing various concentrations of magnesium (0.0–486 ppm) in blackened 2-L glass containers with paraffined plaster of Paris lids. Analytical grade salts without preliminary purification were used in the stock solutions. In order to supply a 486-ppm level of magnesium it was necessary to use a modification of the nutrient solution of Hoagland and Snyder (3). The composition of the nutrient solutions is listed in Table 1. In addition to the salts listed in Table 1, 1 ml of an A_5 (1) micronutrient stock and 1 ml of a 0.5% ferric tartrate solution were added per L of complete nutrient.

Results.—*Host response to magnesium nutrition.*—The growth of tobacco plants lacking adequate magnesium was markedly reduced, varying directly with the supply provided (Fig. 1-A). Severe deficiency symptoms developed on plants at the lower magnesium levels, but concentrations as high as 486 ppm caused neither toxicity symptoms nor pronounced reductions in growth.

Tobacco plants at the 0.0 and 0.5 ppm levels developed severe deficiency symptoms soon after being transplanted. Signs of deficiency appeared first on the lower leaves and later developed successively up the plant. After a time, entire leaves became chlorotic except the midrib and larger veins, which appeared as a green network against the pale background of the laminae (Fig. 1-B). Some affected leaves later developed small necrotic areas and an inward rolling of the lateral veins. This resulted in a downward curling of the leaf margins (Fig. 1-B). Leaves near the tops of plants remained green, though slightly chlorotic. Plants grown with 5.0 ppm magnesium developed deficiency symptoms only on the lowest leaves, without further progress before the end of each experiment.

Magnesium deficiencies markedly reduced the growth rates of tobacco. Plants with severe deficiencies continued to grow throughout each experiment, but much more slowly than plants with adequate magnesium. Plants at 0.0 ppm showed incipient deficiency symp-

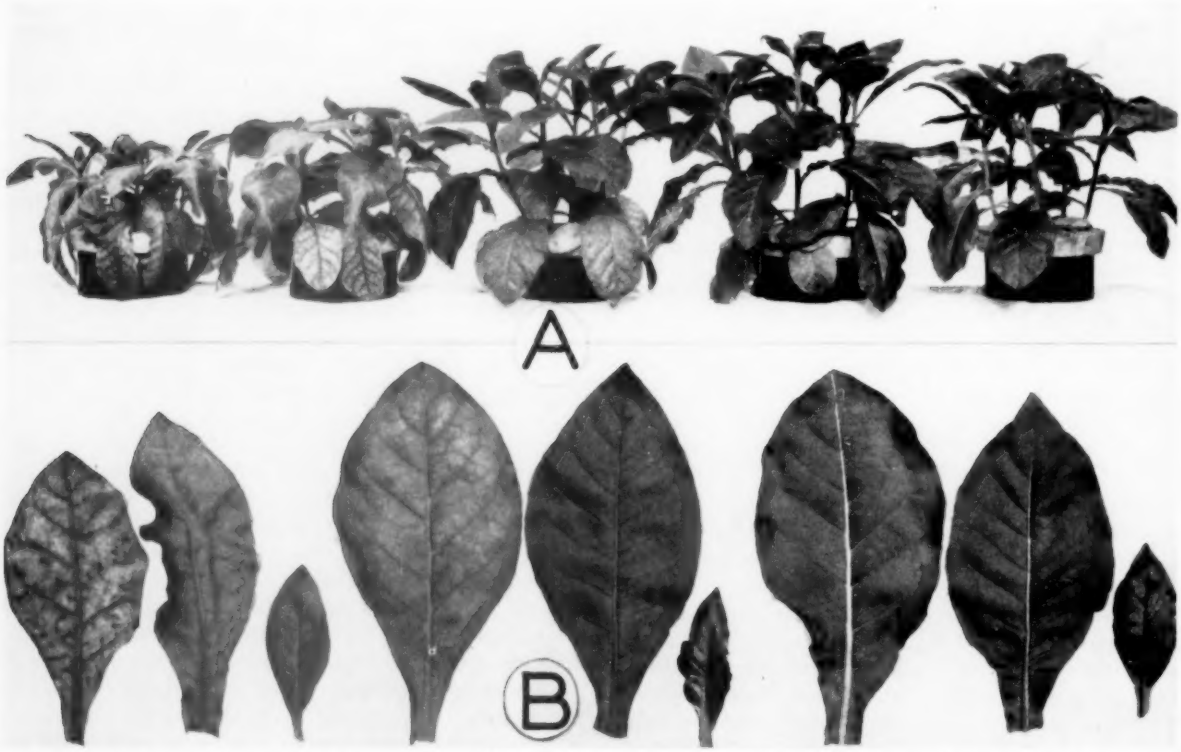

Fig. 1. Influence of magnesium nutrition on TMV-infected tobacco. **A)** Growth after 4 weeks in water culture containing (left to right) 0.0, 0.5, 5.0, 48, and 486 ppm magnesium. **B)** Appearance of lower, center, and tip leaves of plants grown with 0.0 (left), 5.0 (center), and 486 ppm magnesium (right).

TABLE 2.—*Influence of magnesium nutrition of Nicotiana tabacum on TMV multiplication and host growth*

Days after inoculation	Magnesium concentration (ppm)	Av. wet weight whole plants (g)[a]	Virus concentration	
			Local[b] lesions	Optical[c] density
7	0.0	10	16	0.16
	0.5	15	51	0.19
	5	16	89	0.23
	48	18	92	0.25
	486	15	71	0.18
14	0.0	14	213	0.45
	0.5	19	234	0.57
	5	24	297	0.73
	48	23	357	0.65
	486	20	291	0.72
21	0.0	17	417	0.70
	0.5	25	526	0.88
	5	41	491	0.77
	48	40	468	0.81
	486	37	458	0.75

[a] The average of 5 infected plants grown at each magnesium concentration.

[b] The average number per half leaf from 12 half-leaves in an incomplete block.

[c] The concentration of the purified virus in this case was 1/7 the concentration in extracted sap.

toms about 5 days after being transplanted, and growth data taken 1 week later (7 days after inoculation) showed severe stunting compared to plants at the optimum level (48 ppm). Growth was somewhat more rapid at 0.5 ppm than at 0.0 ppm, though the plants were severely stunted compared to those at the higher levels (Table 2).

Virus concentration.—Tobacco plants deficient in magnesium had a lower virus concentration than plants adequately supplied. The differences, however, were not generally great, and a decrease in virus concentration was evident only in severely deficient plants.

Four different experiments were made with levels of magnesium in the nutrient solution ranging from 0.0 to 486 ppm. Both ultraviolet absorbencies of purified virus preparations and infectivity assays with the expressed saps of whole plants consistently showed less virus in severely deficient plants (Table 2). Virus concentration in the expressed saps of the most severely deficient plants (0.0 ppm level) increased consistently during the 3-week period following inoculation, but seldom reached a concentration equal to that in nondeficient plants. There was generally more virus in plants grown with 0.5 ppm magnesium than in plants at the 0.0 ppm level, but less than in plants grown with higher levels of the element (Table 2). Three weeks after inoculation, the expressed saps of plants at the 0.5 ppm level frequently showed as much virus as nondeficient plants, and in some experiments —particularly during the summer, when temperatures were somewhat higher—the differences between deficient and nondeficient plants were scarcely significant at any period after inoculation.

The virus concentration of plants grown with 5 ppm magnesium was seldom significantly different from that of plants at the optimum level (48 ppm), though these plants developed deficiency symptoms toward the end of each experiment. The TMV concentration of plants at the 486-ppm level was not generally significantly different from that of plants at the optimum level.

Virus assays made on a tissue dry-weight basis (8), in general, confirmed the results with expressed saps (Table 3). These assays also represented whole plants, and the results can be compared with those using expressed saps. The virus concentration was consistently lower in severely deficient plants, but the differences were small, showing that TMV can multiply rapidly in plants severely deficient in magnesium.

Discussion.—The effect of micronutrients on virus multiplication probably is in most cases an indirect effect, a result of the function of the element in host cell metabolism. Except for sulfur, none of the micronutrients essential for plant growth are known with certainty to be constituents of plant viruses. Loring and Waritz (5) reported that several minor nutrient elements essential for plant growth, including magnesium, are very tenaciously bound to highly purified TMV. It remains to be seen whether these elements are truly components of the virus.

The mechanisms by which magnesium deficiencies of tobacco limit TMV multiplication could be several in number. In fact, it seems unusual that deficiencies of the element do not have a greater effect on TMV multiplication, considering the various functions of magnesium in the host plant that are probably important in the synthesis of virus components.

Magnesium, in addition to being a component of the chlorophyll molecule, is known to function as an activator of a great number of enzymes. This is undoubtedly one of its most important functions in the plant. Several of these enzymes are active in protein and carbohydrate metabolism (6). Many of the enzymes activated by magnesium can also be activated by

TABLE 3.—*Virus concentration in dry leaf-disc samples from plants grown at various levels of magnesium*

Days after inoculation	Magnesium concentration (ppm)	Per cent dry weight	Grams virus per gram dry tissue
7	0.0	11.7	0.016
	0.5	12.4	0.020
	5	10.0	0.033
	48	9.3	0.028
	486	10.0	0.030
14	0.0	13.5	0.038
	0.5	12.9	0.036
	5	10.9	0.052
	48	10.0	0.071
	486	11.0	0.058
21	0.0	13.0	0.050
	0.5	13.5	0.046
	5	12.8	0.061
	48	12.2	0.061
	486	12.3	0.066

other divalent metallic ions. However, the enzymes involved in adenosine triphosphate-catalyzed reactions, which are responsible for a large proportion of all known biosynthetic systems, show an absolute requirement for magnesium ions (4). Thus, the element might be expected to function in several different systems that participate in TMV synthesis.

The energy-providing mechanisms of the plant, upon which both virus synthesis and host growth are dependent, are undoubtedly impaired by magnesium deficiencies. Most syntheses in the host cell depend on energy available through coupled reactions involving adenosine triphosphate or one of the polyphosphates in which it is in equilibrium. Both the formation and utilization of these energy-yielding compounds would be repressed by magnesium deficiencies, either by way of limiting respiratory substrate through its function in photosynthesis, or in the action of the element as an enzyme activator. Plant growth is limited by magnesium deficiency to a greater relative degree than is multiplication of TMV, indicating that the virus is able to compete successfully with the host for the components and energy necessary for synthesis.

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ELECTROPHORETIC AND IMMUNOCHEMICAL STUDIES OF PROTEINS IN CABBAGE INFECTED WITH FUSARIUM OXYSPORUM F. CONGLUTINANS

R. Heitefuss, D. J. Buchanan-Davidson, M. A. Stahmann and J. C. Walker

Present address of senior author: Institut fuer Pflanzenpathologie, Universitaet Goettingen, Nikolausberger Weg 5A, Goettingen, Germany.

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SUMMARY

Protein extracts of resistant Jersey Queen and Susceptible Early Jersey Wakefield cabbage seedlings, uninoculated and inoculated with the yellows pathogen, were subjected to electrophoretic and immunochemical analysis. By electrophoresis in starch gel, 4 components were separated but no significant difference in resistance or infection could be shown. In contrast, immunochemical analysis with rabbit antisera revealed up to 7 components in extracts of infected susceptible cabbage, compared with 4 components in susceptible healthy, resistant healthy, and resistant inoculated plants. The additional compounds were not original fungus protein. They may have been formed either by the fungus after infection or, more likely, by the infected plant cell. On the basis of different immunological experiments it is suggested that these components are not merely breakdown products but are antigenic substances, probably proteins, that differ from those normally present in the healthy plant.

Introduction.—Although considerable research has been conducted in recent years on the metabolism of host plants in response to invasion by pathogens, very little is known about the role of proteins in the host-parasite interaction. Gassner and Franke (10), Gassner and Hassebrauk (11), and Grümmer (12) attempted to correlate changes in nitrogenous compounds during infection with host resistance and susceptibility, but methods then available did not permit an estimate of changes in the number and kind of proteins. Rohringer (18) reported differences in free amino acids between healthy wheat seedlings and seedlings infected with *Puccinia graminis* Pers. f. *Sp. tritici* (Ericks. & E. Henn.), but no change was found in the bound amino acids after protein hydrolysis. Also, no specific changes in free amino acids due to the action of the parasite could be detected in tomato plants or cuttings after infection with *Fusarium oxysporum* Schlect. f. *lycopersici* Sacc. (20). Andal and Subba Roa (3) found slight differences in rice plants after infection with *Fusarium moniliforme* Sheldon. Barret and McLaughlin (5), using chromatography and electrophoresis, studied the protein fractions of resistant and susceptible wheat seedlings before and after infection by *Puccinia triticina*. They found in infected plants proteins that had a higher electrophoretic mobility than those in healthy plants. Small

additional electrophoretic peaks were also noted in extracts of the infected susceptible variety. In a study of protein metabolism in sweet potatoes infected by *Ceratocystis fimbriata* Ell. & Halst., Akazawa, Uritani, and co-workers (1, 2, 21) found an increase in protein and a decrease in proteolytic activity in the healthy tissue immediately adjacent to invaded tissue. A distinct difference in the electrophoretic pattern showed in uninvaded cells adjacent to diseased tissue a protein that was not present or was very sparse in other healthy tissue.

The present investigation is concerned with the cytoplasmic proteins of resistant and susceptible cabbage plants before and after inoculation with the yellows pathogen, *Fusarium oxysporum* Schlect. f. *conglutinans* (Wr.) Snyd. & Hans. In addition to analysis by electrophoresis, methods of immunochemistry were employed. The latter are the most specific ones now used in protein analysis though they have been used very little with plant proteins except for systematic studies reviewed by Moritz (17). The tendency of plant proteins to precipitate on standing and to show nonspecific reactions with serum frequently complicates and severely limits the application of immunochemical methods to their analysis. However, Rohringer and Stahmann (19) eliminated the non-specific reactions of tomato leaf proteins with normal rabbit serum by isolating the γ-globulin fraction of the serum, and Heitefuss, Buchanan-Davidson, and Stahmann (14) improved the stability of cabbage proteins by extraction and dialysis with buffered glucose solutions. The addition of glucose apparently prevented aggregation and precipitation of the extracted proteins and facilitated a more effective separation of different components by electrophoresis. The stability thus attained was particularly important for this investigation since it was necessary to preserve extracts over periods of several months during which rabbits were being injected and immunological tests carried out. The present study is a first attempt to apply these immunochemical techniques to a study of protein metabolism in relation to plant disease development.

Methods and materials.—The cabbage varieties used were Early Jersey Wakefield (EJW) and Jersey Queen (JQ). They were chosen because they are essentially similar in horticultural characteristics and differ chiefly in resistance to the yellows pathogen. JQ is homozygous for the single gene pair (*RR*) responsible for resistance, whereas EJW is homozygous for the recessive allele (*rr*) that controls susceptibility. Plants were grown in compost soil in a greenhouse at 21°C (±2°). Three-week-old seedlings were inoculated by dipping the roots in a washed spore suspension of the pathogen obtained from a 3-day-old shake culture in Czapek's medium. The inoculated and uninoculated plants were replanted in soil. After 18 days symptoms were evident in the susceptible variety as veinclearing, chlorosis, and stunting. Susceptible plants showing the earliest signs of disease in the form of veinclearing and slight yellowing were cut just above the first leaf. Comparable samples were collected from uninoculated EJW plants and from inoculated and uninoculated JQ plants. No symptoms ever appeared on the resistant JQ plants.

Extraction of plant proteins.—Extraction and separation of the cytoplasmic protein made use of a modification of the method of Wildmann and Bonner (6, 24). All steps were carried out in a cold room at 4°C. First, 100 g of plant material were passed through a meat grinder. The resulting sap and macerate were immediately mixed with 140 ml of cold extraction medium consisting of 0.05*M* phosphate buffer, pH 7.5, 12.5% glucose, and 1 part in 5000 of merthiolate. This mixture was passed several times (total period, 5 minutes) through a water-cooled Eppenbach colloidal mill, final gap setting 4, with intervals of about 15 seconds to avoid heating. A rubber tubing extension to the overflow reduced foaming as much as possible. The resulting pulp was pressed through a double layer of cheesecloth, and the extract was centrifuged for 30 minutes in a Servall centrifuge at 17,000 G. The supernatant was rapidly frozen in dry ice and ethanol, after which it was slowly thawed and centrifuged again for 20 minutes at 20,000 G. The pH after extraction was 6.5. The clear, slightly green extract was dialyzed 24 hours against several changes of the extraction medium. After centrifugation for 20 minutes at 20,000 G the samples were stored in the deep freeze for further use. Aliquots of the nondialyzed and dialyzed extracts were used for determining by Nessler's method (23) the nitrogen soluble and insoluble in 0.5*M* trichloroacetic acid. The total nitrogen and soluble nitrogen (after exhaustive extraction with hot ethanol) were determined from dried aliquots of each sample.

Preparation of fungus mycelium extract.—Shake cultures of the pathogen were incubated 12 days in Czapek's medium. The mycelium was separated from the culture liquid by means of a Buchner funnel and washed several times with water. A total of 17.6 g air-dried mycelium was then ground in a mortar with 50 ml extraction medium. After 4 hours in the cold room the macerate was passed through cheesecloth and extracted again with 20 ml buffered glucose. The combined extracts were centrifuged 30 minutes at 20,000 G and passed through a sterile Seitz filter. After dialysis against the extraction medium, aliquots were used for nitrogen determination and the extracts were stored in a deep freeze.

Electrophoresis in starch gel.—Electrophoresis in starch gel was done by the method of Smithies (22). First, 6.25 g of hydrolyzed starch (Connaught Medical Research Laboratories, Toronto, Canada) was mixed with 50 ml borate buffer (0.0205*M* boric acid + 0.082*M* sodium hydroxide, pH 8.5), and the mixture was heated over a moderate flame until a clear, viscous liquid formed. It was then deaerated under low vacuum for about 20 seconds and poured into plates of 2 × 0.6 × 25 cm. After 20 minutes for solidification, a slit 2.5 mm

wide was cut for insertion of the sample. About 0.25 ml protein extract was mixed with 250 mg hydrolyzed starch and poured into the slit. The excess liquid was removed by means of paper tissue. The starch layer was covered with a thin film of white mineral oil and the sample covered in addition with a cover slip. Strips of filter paper on both ends, for insertion into the buffer reservoirs, were held in place by a cover of Plexiglass, and the whole plate was wrapped in Cellophane. Electrophoresis was carried out at 6 V per cm for 7 hours at 4°C. The electrode compartments contained 0.1*M* borate buffer, pH 8.5. After electrophoresis the starch gel strips were cut in half horizontally, stained 2 minutes in amido black solution, and washed in a 5:5:1 (v/v) mixture of methanol, water, and acetic acid. The gel strips could be stored in this mixture for several months.

Method of immunization.—Preliminary experiments had shown that stronger antisera were obtained by single injections of cabbage protein extracts, using adjuvants, according to the method of Freund (8), than by administering aluminum–ammonium-sulfate–precipitated antigen and employing a larger number of intravenous injections (15). The Freund technique was therefore used. Samples of normal serum were first obtained from each rabbit before beginning the immunization. The injection mixture was prepared by adding 1 part of the protein solution to 1 part of the adjuvant, which consisted of 85 ml of "Bayol F" (light paraffin oil, Stanco Distributors, New York) and 8.5 mg heat-killed *Mycobacterium phlei*, both of which had been mixed by grinding in a mortar, autoclaved, and combined with 15 ml autoclaved "Arlacel A" (Mannide monooleate, Atlas, Wilmington, Delaware). The protein solution and the adjuvant were emulsified by repeatedly forcing both up and down in a syringe. The emulsion was ready for injection when a drop falling onto water from a height of 5 cm did not spread on the surface.

Amounts of 12.5 mg protein in 6.9–9.1 ml emulsion were injected subcutaneously between the shoulder blades of rabbits. Three rabbits for each of the 5 different samples were immunized, and 1 additional rabbit as a control was injected with corresponding amounts of extraction medium and adjuvant. The first test bleeding was done 5½ weeks after injection. At this time, however, the antisera were not sufficiently strong, so that a second injection was necessary. Therefore, 6 weeks after the first injection 2 rabbits of each sample were injected with half the amount of protein and adjuvant originally used. At 8 weeks the remaining rabbit of each sample—previously selected as having the strongest antiserum—was injected with half the original amount of protein without adjuvant. A second test bleeding was carried out after 9 weeks. The rabbits were bled sterilely and sacrificed at 10 weeks. The antisera were stored in a deep freeze for further use.

Antigen-antibody tests.—Normal sera and antisera were tested by ring tests and precipitation tests in agar gel in both tubes and plates (8, 9, 15). The ring tests were not entirely satisfactory, since the antigen in buffer + glucose intermixed very easily with the antiserum and no clear meniscus could therefore be preserved. The tests by single diffusion in agar gel in Oudin tubes did not give good separation of different precipitation lines, for they became rather diffuse upon standing. Therefore the agar diffusion method on plates, which has considerable advantages over the first 2 methods, was used. It is essentially a double diffusion method, in which antigen and antiserum diffuse toward each other through a layer of agar. The main advantage is that in Ouchterlony plates one antigen can be reacted and compared with several antisera, or vice versa, at one time. By selecting a suitable geometric system and equivalent concentrations of antigen and antibody, the precipitation reaction occurs at about half the distance between the reservoirs for antigen and antiserum. If these precipitation lines of one antigen-antisera combination form a continuing arc with those of the adjacent combination, it can be concluded that the antigen compounds are immunologically identical. If they intersect and cross each other without arcing, the precipitating systems are not related.

The cabbage protein solutions were dialyzed against tris acetate buffer, 0.05 ionic strength, pH 7.5, to which 1/5000 merthiolate and 12.5% glucose were added. Agar medium (1.5%) was prepared in the same buffer without glucose. The merthiolate was added to prevent bacterial contamination. A layer 4 mm thick was poured either onto glass plates (5×7-in. photographic plates) or into Petri dishes. After the agar had solidified, holes of 7 mm diameter were cut with a corkborer. The distance between the center of the holes for antigen and antiserum was 2 cm. In some experiments a hole 8 mm in diameter was used for the antiserum in order to increase its amount in proportion to that of the antigen.

The holes were filled with protein extract, normal

TABLE 1.—Nitrogen content of inoculated and uninoculated resistant and susceptible cabbage plants, and of corresponding extracts and protein solutions prepared from them and from mycelium of the yellows pathogen

Source of sample	Dried plant tissue: Total nitrogen (μg/100 mg)	Dried plant tissue: Soluble nitrogen [b] (μg/100 mg)	Crude extract: Total nitrogen [c] (μg/ml)	Dialyzed protein extract: Protein nitrogen [d] (μg/ml)
Uninoculated EJW [a]	5680	765	892	552
Inoculated EJW	5460	820	804	438
Uninoculated JQ [a]	5820	675	876	576
Inoculated JQ	5860	635	908	504
Mycelium of pathogen			119	19

[a] EJW = susceptible Early Jersey Wakefield cabbage; JQ = resistant Jersey Queen cabbage.

[b] Soluble in hot ethanol.

[c] Extracted by 0.05*M* phosphate buffer containing 12.5% glucose.

[d] Trichloroacetic acid insoluble after dialysis of extract.

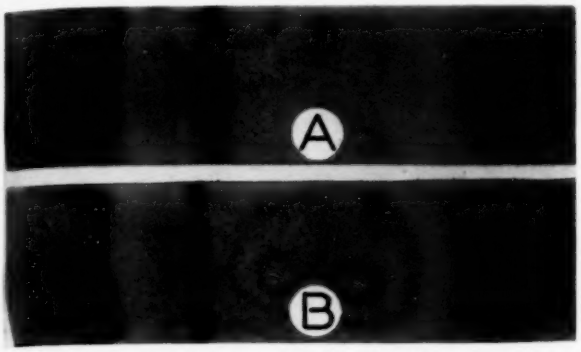

Fig. 1. Electrophoretic separation of cabbage protein extracts on starch gel. Sample placed at left side; migration toward anode. **A)** extract from healthy susceptible Early Jersey Wakefield cabbage. **B)** extract from infected susceptible Early Jersey Wakefield cabbage.

serum, or antiserum. The plates were placed along with wet paper in a plastic container that was tightly closed to secure high humidity and to prevent evaporation of the solutions. The plates were kept in a constant-temperature room at 21±1°C. After 7 days the precipitation lines were evaluated and photographs taken.

Estimation of the amount of antibody produced by the cabbage protein extracts in rabbits was made by the method of Heidelberger and Kendall (13) as modified by Buchanan-Davidson et al (7). Increasing amounts of cabbage protein in 0.5 ml 0.05*M* phosphate buffer + 12.5% glucose were reacted with 0.1 ml antiserum. After 24 hours under refrigeration followed by centrifugation for 30 minutes at 2500 G, the supernatants were carefully decanted and used for estimation of the equivalence point. This was done by ring tests, in which the supernatants were overlaid with antiserum or the protein extract. At the equivalence point neither antigen nor antibody can be detected in the supernatants. The precipitates were washed with 3 ml cold saline, centrifuged, decanted, and drained. The nitrogen in these precipitates was determined by Nessler's method (23). To obtain the amount of antibody nitrogen precipitated, the protein N added was subtracted from the total nitrogen precipitated.

Experimental results.—Table 1 presents the results of nitrogen analysis of dried samples of the plant materials used for protein extraction, of the crude extracts of these samples and the pathogen mycelium, and of the dialyzed extracts used for immunization. The dry matter was about 7.5% of the fresh weight in each of the 4 plant samples. The inoculated EJW plants showed slightly lower total N content, and 7.2% higher soluble N, than the uninoculated EJW plants. The difference was still greater in the crude extracts, with which total N was about 10% less in inoculated than in uninoculated EJW susceptible plants. The protein nitrogen in the dialyzed extracts from the inoculated EJW plants was about 20% less than that of the corresponding uninoculated plant tissue. The differences between the two JQ samples, with which infection and symptoms did not follow inoculation, were relatively small. The results showed that an appreciable effect upon nitrogen metabolism followed infection of susceptible plants.

Starch gel electrophoresis.—The dialyzed extracts of all samples were subjected to electrophoresis in starch gel. Separation into 4 components was distinct, as shown in Fig. 1. Two bands were very dense, and the other 2 very faint. All 4 samples showed nearly the same electrophoretic characteristics. No difference was observed between the 2 major components, and slight differences were noted in the 2 minor components. Both minor lines were present in the extract of healthy susceptible EJW plants, but barely visible in the extract of inoculated EJW plants. Their intensity was also very low in both the healthy and inoculated resistant plants. Since these differences were very small and apparently due to slightly lower protein concentration in those extracts, they were not considered significant.

Analysis of antisera.—Testing the antisera in Oudin tubes revealed strong antibody-antigen reactions after the 10-week immunization period. Antisera were slightly stronger from the rabbits that received protein extract without adjuvant at the second injection. Only some normal sera showed slight turbidity after reaction with the plant protein solutions; however, no turbidity or precipitation occurred at all when normal serum of each rabbit was reacted against the corresponding protein extract on agar gel diffusion plates. It was therefore not necessary to prepare the γ-globulin fraction or to use other purification methods to eliminate nonspecific reactions. Further, no precipitation took place in the control system, which consisted of extraction medium (phosphate buffer + glucose) and its homologous antiserum. This meant that all precipitation lines obtained in the other systems were due to a specific reaction of antibodies produced against compounds present in the plant material injected.

A quantitative estimation of antibody N formed was carried out with only 1 system as a representative sample. Selected for this was the extract of inoculated resistant JQ containing 504 μg protein N per ml, which coincides closely with the average of the 4 protein solutions. The homologous antiserum was that of a rab-

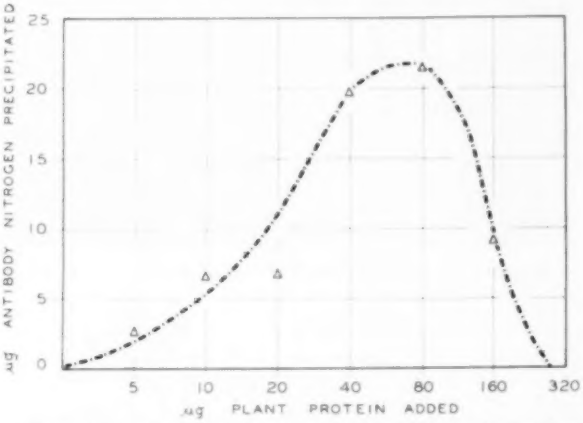

Fig. 2. Antibody nitrogen precipitated from increasing amounts of cabbage protein and 0.1 ml antiserum.

bit, the normal serum of which gave no sign of turbidity with its antigen. Increasing amounts of antigen from 0 to 1280 μg protein in 0.5 ml phosphate buffer + glucose were added to 0.1 ml antiserum. The curve (Fig. 2) indicates the amount of antibody nitrogen present, calculated by subtracting the cabbage protein N added from the total N precipitated. A maximum of 21.6 μg N per 0.1 ml antiserum appears at 80 μg cabbage-protein added, which means that the antibody titer was about 216 μg antibody N per ml antiserum.

Since this curve represents a precipitating system with several components, the amount of antibody N calculated is only an approximation of the true value. It does, however, indicate that the antibody concentration is sufficiently high to perform immunological tests. The equivalence point was between 80 and 160 μg protein added per 0.1 ml antiserum as estimated by reacting antiserum with the supernatant obtained after separation of the precipitated antigen-antibody complex.

Agar gel diffusion.—Very good lines of precipitation were obtained on reaction of the protein extracts against their homologous antisera, as shown in Fig. 3. However, the number of lines differed among individ-

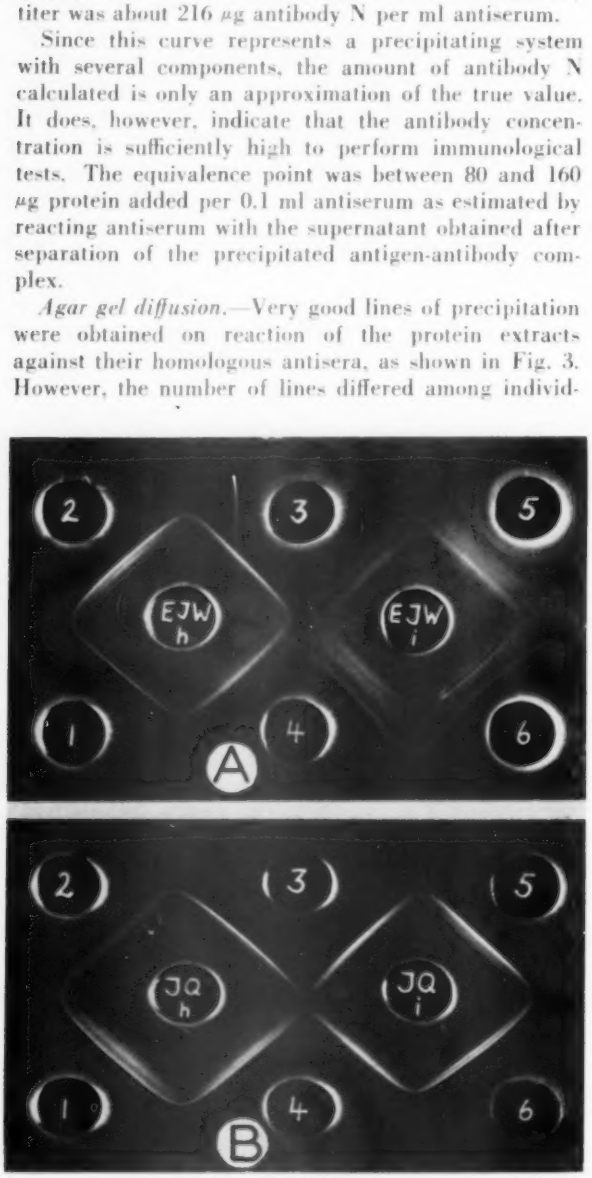

Fig. 3. Agar gel diffusion plates of cabbage protein extracts and their homologous antisera. Center holes contain protein extract; surrounding holes contain antisera obtained from different rabbits. **A)** EJW h = extract from healthy susceptible EJW cabbage; 1,2,3 = homologous antisera; EJW i = extract from inoculated susceptible EJW cabbage; 4,5,6 = homologous antisera. **B)** JQ h = extracts from healthy resistant JQ cabbage; 1,2,3 = homologous antisera; JQ i = extract from inoculated resistant JQ cabbage; 4,5,6 = homologous antisera.

TABLE 2.—Number of precipitation lines obtained upon reaction of different rabbit antisera against the homologous protein extracts on agar gel diffusion plates

Source of protein extract	Number of precipitation lines		
	Rabbit 1	Rabbit 2	Rabbit 3
Uninoculated EJW [a]	3	3	2
Inoculated EJW	7 [b]	5	7 [b]
Uninoculated JQ [a]	4	3	4
Inoculated JQ	4	3	4
Mycelium of pathogen	2 [b]	1	2 [b]
Buffer + glucose	0		

[a] EJW = susceptible Early Jersey Wakefield cabbage; JQ = resistant Jersey Queen cabbage.

[b] One of these lines was weak and not visible in all experiments.

ual rabbits immunized with the same extract, as listed in Table 2. This result is not surprising, since individual animals vary considerably in their ability to form antibodies. These are being formed not necessarily against the maximum number of components present in the antigenic solution but rather against a minimum number. It is therefore justifiable to consider the antisera that reveal the largest number of components present as most characteristic for the corresponding system.

The most striking result of these tests is the larger number of components appearing in the infected susceptible variety. Whereas only 3 lines of precipitation were formed with protein extracts of healthy EJW plants, up to 7 precipitation lines occurred with inoculated EJW. Though these lines were of lower intensity and some were slightly diffuse, there was no doubt about their significance. In addition, a slight difference appeared between the 2 cabbage varieties. Both uninoculated JQ plants showed 1 more antigenic component present than did the uninoculated susceptible EJW. The additional line, however, was not very strong.

Each protein extract was tested against the homologous and heterologous antisera by reacting 1 extract against 5 different antisera on one Ouchterlony plate. The results, summarized in Table 3, revealed that antibody-antigen reactions occurred between all systems with the exception of the fungus extract. At least the 2 main lines of the plant protein systems joined in form of an arc at the point of junction with each other, clearly indicating that the antigenic substances were immunologically identical in all 4 plant extracts. This joining was not clearly established with the minor lines, which did not extend far enough to reach each other. However, the fact that a precipitation occurs between one extract and each heterologous antiserum indicates that antibodies were formed against identical antigenic components.

The fungus extract showed a precipitation line only with its homologous antiserum, but no reaction with antisera against infected or healthy plants. In addition, no reaction occurred between the antiserum against fungus mycelium and all plant extracts. The antigenic components of the fungus mycelium are therefore not present in the protein extract of infected plants.

The additional lines formed upon reaction between

TABLE 3.—Interactions of homologous and heterologous antigen-antiserum systems as indicated by number of precipitation lines obtained on agar gel diffusion plates

Source of protein extract	Antiserum: EJW Uninoculated	EJW Inoculated	JQ Uninoculated	JQ Inoculated	Mycelium of pathogen
Uninoculated EJW[a]	3[b]	4[c]	4	4	0
Inoculated EJW	3	7[c]	4	4	0
Uninoculated JQ[a]	4[c]	4[c]	4	4	0
Inoculated JQ	4[c]	4[c]	4	4	0
Mycelium of pathogen	0	0	0	0	1

[a] EJW = susceptible Early Jersey Wakefield cabbage; JQ = resistant Jersey Queen cabbage.
[b] Values are underlined where antigen and antiserum are homologous.
[c] One of the lines was weak and not visible in all experiments.

the extracts of susceptible infected plants and their homologous antisera showed in several cases typical crossing with the lines of the healthy system. In other experiments the shape of the additional lines indicated no sign of bending toward the other system. Such a crossing of different precipitation lines gives positive evidence that they represent antigenically different components.

One example is shown in Fig. 3-A, in which the precipitation line from the infected susceptible system crosses the one from the healthy susceptible system. In contrast, the precipitation lines of both the resistant healthy and the resistant inoculated system clearly join each other, as shown in Fig. 3-B.

Seven precipitation lines (6 in some experiments) were found when extract from inoculated EJW was tested against its homologous antiserum. Only 3 or 4 lines were formed when extract from uninoculated EJW was tested against the antiserum of inoculated EJW. Three lines were formed in the opposite combination, i.e., extract from inoculated EJW tested against antiserum of uninoculated EJW. This indicated that 3 or 4 additional compounds appeared in the extract of inoculated EJW plants, against which no antibodies were present in antisera from extracts of healthy EJW plants.

Four precipitation lines were formed in the homologous systems of uninoculated and inoculated JQ plants. Four lines also appeared when extracts from uninoculated or inoculated EJW were tested against antisera from the 2 extracts of the resistant variety. The fourth line, however, was extremely weak when the 2 protein extracts from resistant JQ plants were reacted with the antisera against uninoculated and inoculated EJW plants.

The results indicate that the difference between the susceptible EJW and resistant JQ variety is only a quantitative one. Antibodies against a fourth component were formed in the antisera to the resistant variety. If this compound is present in the susceptible variety also, but only in a very low concentration, an antibody-antigen reaction can take place between antiserum to the resistant variety and protein extract of the susceptible variety. This was actually demonstrated in the results. A very faint line of precipitation should occur when the extract from resistant plants reacts with the antiserum against the susceptible plants in which antibodies reacting toward the fourth component are apparently absent or very low in concentration. This was the case, since a very weak fourth line appeared in this system also. This leads to the conclusion that the susceptible and the resistant varieties both contain 4 antigenic components, one of which is present only in a low concentration in the susceptible variety EJW. Consequently only 2 or 3 new antigenic components appear in the infected susceptible tissue.

Another possibility of demonstrating the presence of additional components by antibody-antigen reactions is the absorption technique: A sufficient amount of an antigenic mixture A (smaller number of components) has to be added to a second antiserum B (larger number of components) so that, after removal of a precipitate, no further reaction occurs between the "absorbed serum" and antigen A, indicating complete absorption. If a reaction then takes place between the absorbed serum B and its homologous antigen B, it can be concluded that precipitation lines appearing in this system represent additional components present in the antigenic mixture B but not present in the antigenic mixture A.

To clarify the possible difference between the susceptible and resistant variety, 0.5 ml of resistant JQ antiserum was absorbed with 0.25 ml of susceptible EJW protein extract. No further precipitation reaction took place between the absorbed serum and either antigen, indicating that no antibodies were left in the absorbed serum to react with a possible additional fourth compound in the resistant variety. In the reverse system also, in which susceptible EJW antiserum was absorbed with the resistant JQ antigen, no further precipitation occurred with either antigen. In contrast to this, when absorption experiments were carried out with the system of uninoculated susceptible EJW (4 components) and inoculated EJW (7 components), it was not possible to remove all components from the "infected serum." When 0.5 ml antiserum was absorbed with 0.3 ml healthy EJW protein extract, the absorption was complete and antibodies against 3 additional compounds were still present in the antisera from extracts of infected plants. Later attempts to increase the intensity of the remaining lines were not satisfactory, because the antibody concentration apparently was not high enough. Obtaining a stronger antiserum-antigen combination by preparing the γ-globulin fraction of the serum was not feasible, because by that time a precipitate began to form in the plant extract. The fact, however, that the additional lines in the protein extract of the infected plants were not removed in the previously mentioned experiments, indicates that they are new antigenic components appearing after infection.

Discussion.—Though electrophoresis revealed no significant qualitative differences in protein extracts of

healthy and infected cabbage, the immunochemical analysis showed a completely different picture in the form of an increased number of antigenic components present in extracts of infected susceptible plants. It is possible by means of immunological methods to distinguish between extracts that show essentially the same electrophoretic behavior. An ideal system for clarifying this picture would be the combination of electrophoresis and immunology, which has been successfully utilized in several cases (8, 16).

There is no doubt about the presence of these additional components in infected cabbage. By no means can they be artifacts, which appeared only as a result of proteolytic decomposition in the extract during the storage period. In this case more precipitation lines should appear upon reaction of the extract from infected plants against the heterologous antiserum, at least against that of healthy EJW plants. This, however, was not the case.

The question arises as to whether these additional precipitation lines represent new antigenic susbtances present in infected cabbage, or whether they are merely breakdown products of the original protein in healthy plants. This question may be answered by the following considerations. Different antibodies may be formed against different antigenic sites on a protein molecule and appear separately in the resulting antiserum. If, as a result of proteolytic breakdown, smaller units are formed, an antibody-antigen reaction could still take place. But in this case it would reveal the number of smaller reactive units now present (16).

This possibility was checked by reactions of the heterologous systems. The increased number of compounds appeared only in the homologous system where infected EJW protein extract was reacted against antiserum to infected EJW. Not more than 4 "normal" components were detected in any other heterologous antiserum-antigen combination. If the 2 or 3 additional components were merely subunits of the original ones present, the increased number of lines should also appear in the heterologous system of extract from infected plants against antiserum to healthy plants. Such a reaction did not occur.

Another possibility is that, by proteolytic breakdown into subunits of the original molecule, additional new antigenic sites might be exposed. This type of new antibody could not be formed against the original molecule. Precipitation reaction between the newly formed fragment and the antiserum obtained against the original molecule would not occur if this new site were the only one available for antibody antigen reaction. This case, however, is not very likely.

The additional compounds appearing after infection are therefore regarded as antigenically different from those originally present in the healthy plant on the basis of 3 points of evidence: 1) the increased number of components can be demonstrated only in the system of infected susceptible EJW plants against their homologous antiserum; 2) the additional lines show in several cases the typical crossing with those of the "healthy system"; 3) it was not possible in absorption experiments to remove the antibodies against the additional components from "infected antiserum."

Since immunochemical analysis revealed that a strong reaction occurred only between fungus extract and homologous antiserum, but not at all between fungus extract and antiserum from infected plants or vice versa, there is little possibility that the new compounds are cellular fungus proteins. The question remains whether the type and antigenic character of proteins formed by a fungus on a synthetic medium are the same as when this fungus is growing in the host plant itself. The combination of host and parasite might well result in a shift of the parasite metabolism, as it does in the metabolism of the host. The specific interaction of both, which enables the parasite to synthesize certain substances, is extremely important for obligate parasites. It might be equally important for the resistance phenomena in both facultative and obligate parasites. The evidence does not justify a final decision as to whether the newly appearing components after infection are synthesized by the parasite itself, when it is growing on the host plant, but it can certainly be concluded that they are not present in the fungus grown on a synthetic medium.

On the other hand, the infection of cabbage by *Fusarium oxysporum* f. *conglutinans* does induce a very drastic change in the protein metabolism of the host. Though the plants were harvested at an early stage of the disease, with typical symptoms but no sign of wilting, visible symptoms characterize a deeply disturbed metabolism at a point at which the host-parasite relationship is clearly established. Since the pathogen grows not only in the xylem elements but enters living host cells as well (4), the invaded and the surrounding cells might respond by an increased metabolic activity, as has been shown for other plant diseases. The result of this increased activity is not necessarily an increase in the net amount of proteins. In infected cabbage, even less total protein is found. It may, however, induce the formation of new proteins of protein complexes that are not formed in a normal cell. Akazawa, Umemura, and Uritani (2) obtained similar results with sweet potatoes, in which a qualitative difference in the protein fraction was found after infection with *Ceratocystis fimbriata*, as well as an increased amount of protein in the tissue adjacent to the site of infection. They suggested that the parasitic attack may lead to a decomposition and rearrangement of protein molecules, which might in some way participate in the active metabolism of the infected cell. Nothing is known so far about the function that the newly appearing components have in the metabolism of infected cabbage plants. Clarification of this point would be important for future work. The extent to which the plant can counteract the attack of the parasite, not only on the protein metabolism but on the balance of different metabolic processes, will undoubtedly have a bearing on the degree of resistance and susceptibility.

Whereas small quantitative differences were detected, no evidence was found for a qualitative difference

in the protein fraction of uninfected resistant and susceptible cabbage. Such a "preformed" difference is very unlikely to be responsible for resistance. The important role of proteins in relation to resistance is rather to be expected in their function. Certainly a plant contains many more proteins than those that can be distinguished by the present analytical methods. Information about resistance in relation to proteins will be accomplished only if we succeed in revealing as many components as possible of this "mosaic" of protein molecules and their function in regard to the attack of the parasite. Immunochemistry, in connection with other methods, will prove a very powerful tool with which to pursue this question.—Departments of Biochemistry and Plant Pathology, University of Wisconsin, Madison.

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VICTORIA-TYPE RESISTANCE TO CROWN RUST SEPARATED FROM SUSCEPTIBILITY TO HELMINTHOSPORIUM BLIGHT IN OATS

H. H. Luke, H. E. Wheeler, and A. T. Wallace

Respectively, Plant Pathologist, Crops Research Division, U. S. D. A.; Professor of Botany, Department of Botany, Louisiana State University, Baton Rouge; and Geneticist, Florida Agricultural Experiment Station, Gainesville.

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SUMMARY

About 50 million oat seedlings of 2 varieties susceptible to Helminthosporium blight were screened in an effort to separate the Victoria type of crown-rust resistance from susceptibility to *H. victoriae*. The specific toxin produced by *H. victoriae* was used as a screening agent to separate susceptible from resistant seedlings in the 2 populations. Seventy-two (18 Victorgrain and 54 Fulgrain) mutant types that proved resistant to Helminthosporium blight were selected. These selections were tested with 5 races of crown rust, and only 2 exhibited resistance similar to that of the parent variety (Fulgrain). The resistance of these 2 mutants to races 202 and 203 was similar to that of the parent and Victoria; however, their reaction to races 205 and 290 was unlike that of the parent and Victoria. Moreover, these mutants were not only resistant to *H. victoriae* but exhibited the typical Victoria necrotic reaction to 4 races of crown rust. It thus appears that the genetic relationship between crown-rust resistance and Helminthosporium blight susceptibility is not simple, but is conditioned by a number of genes.

Helminthosporium victoriae Meehan & Murphy was first isolated from oats by Meehan and Murphy, in 1944, and by 1946 was known to be present in 19 states (10). The disease caused by this fungus soon became widespread throughout the north-central portion of the United States, and by 1947 was found in most of the oat-growing areas of the United States and Canada (12, 19). The prevalence and severity of this disease soon made it necessary to replace susceptible varieties with varieties resistant to *H. victoriae*. This proved unsatisfactory because the replacement varieties were more susceptible to certain races of crown rust than the Victoria derivatives they replaced.

The disease incited by *H. victoriae* caused severe damage only to certain varieties that carried the Victoria type of crown-rust resistance (7, 8, 12). Therefore, a number of workers initiated studies to determine the mode of inheritance of the Victoria-type resistance to crown-rust and to separate this type of resistance from susceptibility to *H. victoriae*. Certain investigators reported (2, 3, 7, 8, 12, 13, 18) that the Victoria type of crown-rust resistance was conditioned by a single dominant factor, whereas others (6, 15, 16, 19) reported that resistance was governed by more than a single-factor pair. Further evidence indicated that the gene combination for the Victoria type of crown-rust resistance also conditioned susceptibility to *H. victoriae* (2, 7, 8, 12). Until the studies reported here were initiated, these two characters had not been separated. It was believed that the reaction to these two diseases was a pleiotropic effect of a single gene (7, 8).

Conventional genetic techniques apparently failed to break this association and also left some doubt as to the number of genes carried by Victoria for crown-rust resistance. Therefore, the studies reported here were initiated in an effort to determine more clearly the number of genes involved and to study the linkage relationship between the Victoria crown-rust reaction and Helminthosporium blight susceptibility. Studies by other workers indicated that these 2 characters were either closely linked or conditioned by the pleiotropic effect of a single gene. In the former case, the chances of isolating a particular genetic combination would be poor if small populations were studied. Therefore, these studies involved the use of a mass-screening technique designed to select Helminthosporium-blight resistant mutants from susceptible varieties. Such a method would be more likely to succeed than conventional breeding techniques because extremely large populations could be tested. The mass-screening method offers another advantage since the tests could be conducted in 3–4 months instead of the 2–3 years required with conventional genetic techniques.

The screening technique.—The toxin produced by *H. victoriae* and used as a screening agent in this study has been shown to cause the same disease symptoms and to possess the same specificity as the pathogen itself (8, 9, 11, 20). Therefore, the toxin could be substituted for the fungus. This was desirable since the toxin technique permits adjustment of the selection pressure. Moreover, the same degree of selection pressure can be applied in replicated tests. The ragdoll technique described by Ivanoff (4, 5) does not permit adjustment of the selection pressure. The toxin technique eliminates this disadvantage and was used throughout this study.

Fig. 1. Effects of the toxin produced by *H. victoriae* on seedlings of a susceptible variety, Fulgrain. Left: resistant seedlings. Right: susceptible seedlings. Note the somewhat intermediate reaction of the seedling at the extreme left.

TABLE 1.—Crown-rust reactions[a] of 2 blight-resistant mutants compared with those of their parent and Victoria

Rust race	Mutant 56-2	Mutant 280	Fulgrain	Victoria
202	2-N	2-N	2-N	2-N
203	1-N	1-N	1-N	1-N
205	1-N	1-N	0-CN	0-CN
290	1-N	1-N	0-C	0-C
216	4	4	4	4

[a] Numerals designate conventional infection types, while N = typical Victoria type of necrosis and C = chlorotic fleck; thus 0-CN designates chlorotic infection type 0 with slight necrosis.

The toxin technique, having been described previously (21), is presented only briefly in this report. The method consisted of germinating oat grains for about 48 hours, or until the roots were up to 10 mm long. Then young seedlings were thoroughly sprayed with a solution containing 10 units per ml of the toxin produced by *H. victoriae*. Details of production and bioassay of this toxin were also presented in another paper (9). The treated seedlings were maintained under moist conditions for about 72 hours and then examined for individuals that had survived. Following the initial test, survivors were heavily inoculated with the fungus; therefore, any susceptible seedlings that escaped the toxin test were eliminated.

Results of the mass-screen.—Fifty bushels of Victorgrain 48-93 and 50 bushels of Fulgrain (about 50 million seedlings) were screened, and 502 seedlings survived the toxin treatment as well as heavy inoculations with *H. victoriae*. Before inoculation with the fungus, a few seedlings exhibited intermediate reaction to the toxin treatment. (See seedling at the extreme left of Fig. 1.) These seedlings exhibited growth inhibition and slight browning of root tips, but did not show the severe necrosis shown by completely susceptible types. Many of these intermediate types survived 12–14 days longer than Fulgrain when heavily inoculated with *H. victoriae*. Observations of plant and grain characters for 3 years indicated that 54 of the original 502 selections were Fulgrain types and 18 were Victorgrain 48-93 types. In repeated tests with *H. victoriae* and crown rust, none of the 72 selections segregated for reaction to either disease. The fact that these lines did not segregate and were identical to the parent (except for disease reaction) indicates that they originated as mutants rather than from outcrossing. Since the remaining 430 selections differed in agronomic type from their putative parents they were assumed to be outcrosses or mechanical mixtures of other varieties.

Crown-rust tests.—After the mutants had been separated from the varietal mixtures, they were screened with race 202 of crown-rust to determine whether blight susceptibility had been separated from crown-rust resistance. Of the 72 mutants isolated, only 2 possessed rust resistance similar to that of the parent. In the initial tests, conducted in 1956 in the greenhouse, it was difficult to determine whether the crown-rust resistance of the mutant selections was identical to that of the parent; or only similar. Therefore, a series of tests were initiated to answer this question.

Victoria and varieties carrying the Victoria type of crown-rust resistance exhibit a range of infection types to various crown-rust races. Therefore, races 202, 203, 205, 290, and 216 were selected to represent the range of infection types. Races 202 and 203 were differentiated with the new set of differentials and are respectively very similar to or the same as races 45 and 57. Victoria is susceptible to race 216, but exhibits varying degrees of resistance to the other 4 races.

In order to compare the infection type of the 2 blight-resistant mutants with that of Fulgrain and Victoria, a code was developed to designate identical or similar reactions (see footnote, Table 1). Such a system is necessary for work with the Victoria type of rust reaction, since conventional readings may not indicate whether reactions are identical or merely similar. For example, Victoria may exhibit infection-type 1 with severe necrosis while Appler inoculated with the same race may also exhibit infection type 1 without necrosis; thus the two reactions, though quite different, fall in the same infection class.

Results of crown-rust tests.—All mutants were tested with the 5 races mentioned above, and only 2 proved to carry resistance to 4 of the 5 races. The rust reactions of these 2 mutants are compared with those of victoria and the parent in Table 1 and illustrated in Fig. 2.

These results show that the 2 blight-resistant mutants, Fulgrain, and Victoria all react similarly to races 202 and 203. However, the latter exhibited a higher type of resistance to races 205 and 290 than did the mutants. The reaction of the mutants to races 205 and 290 was very similar to the reaction of Fulgrain and Victoria to race 203 in that they showed necrotic-infection type 1. Further observations indicated that the mutants carry the same type of resistance to races that sporulate on Victoria and Fulgrain, but do not carry as high a type of resistance to races that do not sporulate on the latter. In addition, all mutants were tested with race 216 and none were resistant.

Discussion and conclusions.—Some of the early reports on the inheritance of the Victoria type of crown-rust reaction suggested that this variety carried 1 dominant gene for resistance completely linked to susceptibility to Helminthosporium blight (3, 7, 8, 12). The physiology of this relationship was discussed by Litzenberger (8) and Paddock (14), who stated that Victoria oats carried a gene that made the plant very sensitive to toxins produced by crown rust and *H. victoriae*. These workers concluded that the gene conditioning toxin-sensitivity also made the Victoria variety susceptible to Helminthosporium blight and resistant to crown rust. That is, the plant was so sensitive to the toxin produced by the crown-rust fungus that the surrounding area soon became necrotic and thus isolated itself in an island of dead tissue. The obligate parasite could not survive in such an environment; therefore, varieties that exhibited this super-

sensitive reaction were resistant. On the other hand, *H. victoriae* is a facultative saprophyte and will survive on dead tissue. Thus the variety was susceptible. A more recent report, by Welsh et al (19), indicated that Victoria appeared to have 3 dominant genes conditioning resistance to race 45 of crown rust. Only 1 of these genes was associated with susceptibility to Helminthosporium blight. These workers concluded that the gene associated with blight susceptibility is the only gene that expresses the Victoria necrotic type of crown-rust reaction (the R— gene of Welsh et al). Fulgrain and Victorgrain apparently have the full complement of genes from Victoria because they are susceptible to blight and respond to races 202 and 203 in the same way that Welsh et al reported for progeny of Garry and R.L. 1987. The latter are both Victoria derivatives susceptible to Helminthosporium blight.

When the mutant lines isolated in the study being reported here were examined for reaction to races 202 and 203, they appeared to have the same complement of genes for rust resistance as their parent in addition to being resistant to *H. victoriae*. However, when these mutants were examined for their reaction to races 205 and 290, they did not appear to have the same genetic complement for rust resistance as Fulgrain and Victoria. When the data in Table 1 and Fig. 2 are examined, it can be readily seen that the mutants, which are blight-resistant, also show the typical Victoria necrotic-rust reaction. Therefore, it appears that mutations have occurred in the gene that governs susceptibility to *H. victoriae*, and by the use of the mass screening method the mutants were isolated. The crown-rust reaction of the two mutants (56-2 and 280) is very similar to that of the Fulgrain parent, but not completely identical. It thus appears the gene that causes the plant to be supersensitive (resistant) to the crown-rust fungus is not the same gene that makes the plant susceptible to the toxin produced by *H. victoriae*.

These observations indicate that the gene reported by Litzenberger (8) to be pleiotropic for Helminthosporium-blight susceptibility and crown-rust resistance may be 2 or more linked genes. Furthermore, instead of Victoria having 3 genes for crown-rust resistance, as reported by Welsh et al (19), this variety may have more than 3 genes conditioning crown-rust resistance and susceptibility to *H. victoriae*. It is possible that the "locus" governing crown-rust resistance and blight susceptibility is a "nest" of closely linked genes inherited as a single segment. This view was expressed by Konzak (6), who suggested that this locus may be a complex locus similar to the "R" locus described in maize by Stadler (17). Additional information on the complexity of this "locus" is that lines of oats have been isolated that show different degrees of resistance to Helminthosporium blight. This view is strengthened by the observations of Atkins (1), who reported that Mustang, a Victoria derivative, was more tolerant to blight than other varieties developed from Victoria parentage. Of course, these speculations need to be confirmed with genetic data, which are now being col-

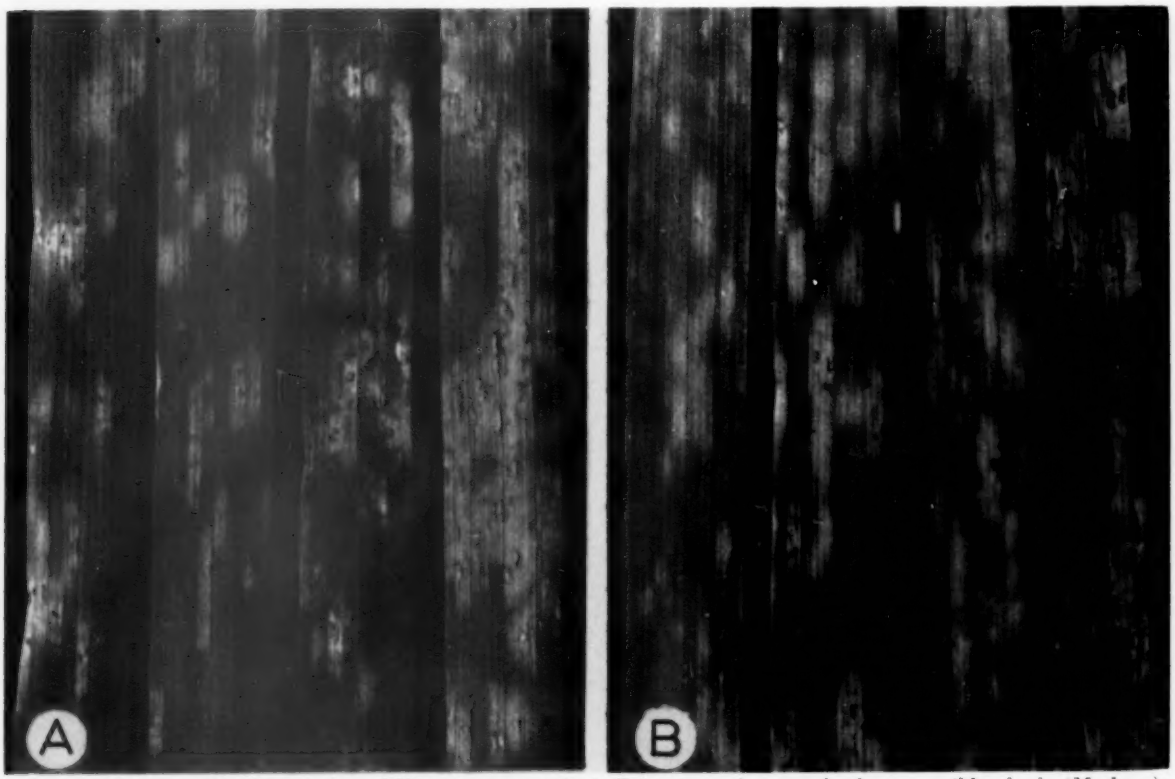

Fig. 2. Left to right, the reaction of Victoria, mutant 56-2, Fulgrain, and a completely susceptible check (Markton): **A)** to crown rust race 202; **B)** to race 290.

lected.—Crops Research Division, ARS, U.S.D.A.; Botany, Bacteriology, and Plant Pathology Department, Louisiana State University, Baton Rouge; and Florida Agricultural Experiment Station, Gainesville.

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HOSTS FOR DIFFERENTIATING OAT LOOSE-SMUT RACES OF THE SOUTHEASTERN UNITED STATES

H. H. Luke, D. D. Morey, and S. J. Hadden

Respectively, Pathologist, Crops Research Division, U. S. D. A., and Plant Pathology Department, University of Florida, Gainesville; Associate Agronomist, Georgia Coastal Plain Experiment Station, Tifton; Plant Breeder, Coker's Pedigreed Seed Company, Hartsville, South Carolina.

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SUMMARY

Over 50 varieties and lines of oats were evaluated as differentials for southern loose smuts. Ten were selected that differentiated satisfactorily 4 distinct collections. Since the data available were not complete enough for a comprehensive comparison between these collections and previously described races, they were tentatively referred to as southern loose-smut groups. Some comparisons, however, were possible, and it appears that 2 of the 4 groups may be new races. In addition, these 10 varieties differentiated races A 14 and A 15 as well as 4 field collections.

In 1924 Reed (6) first demonstrated "host specialization" of the oat loose-smut fungus, *Ustilago avenae* (Pers.) Rostr. His contribution was soon confirmed (11, 12), and by 1930 the concept of host specialization of this pathogen was well established. Since then, many races of oat loose-smut have been reported from various sections of the United States (2, 3, 4, 9, 10, 13, 16), Canada (1), and the British Isles (17). Several of the races identified by U. S. workers originated in the South; thus, the problem of smut races in this area has been recognized for some time.

In recent years, oat varieties with widely different genetic makeup, grown in the South, have perpetuated a number of smut races. Since less than 20% of the oat seed planted in this area is treated, the loose-smut problem has become both complex and acute. Moreover, certain smut collections from the South failed to infect the differentials under field conditions at Pullman, Washington (4), and differential varieties that were successful at Pullman proved unsatisfactory in Florida and Georgia. Therefore, a study of the oat loose-smut problem was initiated in 1955, primarily to select adapted varieties that would differentiate the loose-smut races of the southeastern area, and to survey and identify these races. Such accomplishments should help the oat-improvement program.

Materials and methods.—During the initial phases of this study, the Waring Blendor, partial vacuum, and dehulled-seed techniques of inoculation were evaluated. Percentages of infection were highest with dehulled seed, and this technique was used throughout the study. When possible, 50 dehulled seed were planted in a 5-ft. row or 100 seed in a 10-ft. row. About

TABLE 1.—The average reaction [a] of 10 oat varieties to 4 southern loose smut groups at Gainesville, Florida (G), and Tifton, Georgia (T), over a 4-year period.

Host	C.I.[b] no.	Group 1		Group 2 [c]		Group 3		Group 4	
		G	T	G	T	G	T	G	T
Seminole	5924	0	0	0	0	0	0	0	0
Red Rustproof-14	4876	0	2	3	3	0	0	27	45
Appler	1815	1	2	2	2	1	1	23	17
Victorgrain 48-93	7125	0	0	0	0	0	0	95	58
AB 301	7437	70	22	1	2	0	0	0	0
Atlantic	4599	95	85	96	93	4	8	0	0
Stanton	5116	92	98	98	96	2	3	0	2
Fulghum	708	79	43	0	2	55	50	0	2
Southland	5207	93	68	88	52	33	22	0	1
Wintok × Santa Fe	7230	65	84	52	46	62	54	15	85

[a] The number of smutted panicles per 100. Fewer than 10% smutted panicles is considered a resistant reaction.
[b] C.I. refers to accession number of the Cereal Crops Research Branch, Crops Research Division, U. S. D. A.
[c] Data for group 2 represent a 2-year average.

100 mg of chlamydospores were applied to each seed lot (50 or 100 seed per lot) before it was planted. Seed treated in this manner was planted in uniform plots at Gainesville, Florida, and Tifton, Georgia, over a 4-year period beginning in 1955.

Four different "smut groups" were used. The spore collections were tentatively referred to as groups, since data available were not complete enough to make a comprehensive comparison with previously described races (4, 7). Group 1 originated from collections made in 1943 from Victoria (C.I. 2401), Victorgrain Strain 1 (C.I. 3692), and various other Victoria derivatives in the oat-breeding nursery of Coker's Pedigreed Seed Company, Hartsville, South Carolina. Victorgrain Strain 1 proved to be heterozygous for reaction to this group, and the smut was initially increased and maintained on homozygous-susceptible selections. This collection was maintained on various strains of Stanton until 1952, when it was transferred to Arlington (C.I. 4657). When group 1 was shifted from Stanton to Arlington, the latter variety showed increasing percentages of infection. Apparently Arlington was expressing a screening effect on the biotypic composition of the smut collection, with only the particular biotype or biotypes most pathogenic to Arlington being perpetuated. In an attempt to recover the original biotypic composition thought to have been present in this collection, naturally occurring loose smut was collected from the Coker nurseries in 1955. These collections were from Victoria derivatives, but principally from Fultex (C.I. 3531). These collections were composited, maintained on Stanton (C.I. 5116), and later designated group 2.

The exact origin of group 3 is uncertain, but this collection is known to have been present in the Coker breeding nurseries for at least 25 years. It is likely that the original collection was from Coker Fulghum number 4 (C.I. 3166) or some similar strain. In recent years it has been maintained on Fulghum (C.I. 708).

Group 4 originated as a collection made in 1939 from Fulgrain Strain 1 (C.I. 3253). Before 1950 this group was maintained on Fulgrain Strain 3 (C.I. 3697), and since then on Victorgrain 48-93 (C.I. 5355 and C.I. 7125).

In addition to these 4 smut groups, races A 14 and A 15 (4) were used in the 1957 tests. These 2 races are "Victoria races" and were used in an attempt to identify groups 1 and 2, which exhibit infection patterns similar to those of Victoria races. Four field collections from Georgia were also used in the 1958 test.

Results.—Over 50 selections and varieties of oats were tested to determine those best suited as differentials for southern smuts. Results obtained over a 4-year period are presented in Table 1 and summarized in Table 2.

Certain Victoria derivatives (Atlantic, AB 301, and Stanton) were used to delimit the "Victoria smuts" that appear to be common in the Southeast. Southland and Fulghum are susceptible to the Fulghum-type smuts as well as to certain Victoria smuts and were included with the Victoria derivatives to differentiate these 2 groups. Red Rustproof-14, Appler, and Victorgrain 48-93 were used to differentiate the "Red Rustproof" smuts thought to be present in this area. Seminole was resistant and C.I. 7230 was susceptible to all groups and races tested.

As shown in Table 1, groups 1 and 2 differ from the other 2 in being highly virulent on Atlantic and Stanton. Group 1 differs from group 2 by the resistant reaction of AB 301 to the latter. Group 3 is similar to groups 1 and 2, but is characterized by its ability to parasitize Fulghum and Southland. Victorgrain 48-93 is resistant to all collections other than group 4, which differentiates this group from the other 3.

Data in Table 2 show that the susceptible and resistant reactions of 6 of the 10 hosts differentiate the 4 southern smut groups. They also differentiate races A 14 and A 15 as well as 2 races in 4 field collections from Georgia. One of the latter exhibited reactions similar to those of group 2. The other 3 were the same as group 4. It thus appears that these differentials will separate satisfactorily most, if not all, of the smut races presently known in the southeastern United States.

Discussion and conclusions.—The inconsistent behavior of physiologic races of oat loose smut on a given set of differential hosts has been recognized and discussed by several workers (4, 5, 13, 17). Holton

TABLE 2.—Reaction[a] of 6 oat varieties to 4 southern loose smut groups (S), 4 field collections from Georgia (G), and 2 races (A-14 and A-15)

Smut collections	Wintok × Santa Fe C.I. 7230	Southland C.I. 5207	Fulghum C.I. 708	Atlantic C.I. 4599	AB 301 C.I. 7437	Victorgrain C.I. 7125
S 1	S	S	S	S	S	R
S 2	S	S	R	S	R	R
S 3	S	S	S	R	R	R
S 4	S	R	R	R	R	S
G 1	S	S	–	S	R	R
G 2	S	R	–	R	R	S
G 3	S	R	–	R	R	S
G 4	S	R	–	R	R	S
A 14	S	–	R	–	R	R
A 15	S	–	S	–	R	R

[a] Less than 10% smut is considered a resistant (R) reaction.

and Rodenhiser (4) and Tervet (13, 14, 15) suggested certain techniques that reduced this undesirable variability in some cases, but no one has obtained consistently uniform results with oat smuts under field conditions. In this study, variation was considerable from year to year and from location to location in the same year. Since the study's purpose was to identify and classify races of *U. avenae*, it is not critical to know whether these smuts always incite a similar degree of infection on the differential varieties. This viewpoint appears valid as long as the identity of each group or race can be maintained. From this standpoint, it appears that the primary objective of this study was accomplished.

Comprehensive reports on races of oat smuts were presented by Reed (7) and Holton and Rodenhiser (4). In an extensive study, Reed described 29 races of loose smut, 17 of which were collected in the United States. Holton and Rodenhiser identified 15 loose-smut races found in a wide range of locations throughout the United States. In a more recent report Reed et al (10) identified 2 Victoria races, 1 of which may be related to group 1 used in this study. Since it parasitizes Fulghum and certain Victoria derivatives, group 1 may be similar to race A 30 or A 31, described by Reed et al (10). Group 1 is also very similar to race A 15, described by Holton and Rodenhiser (4). For example, both parasitize Fulghum, Atlantic, and Stanton. Group 1 is distinct from group 2 in that the former is pathogenic on both AB 301 and Fulghum, and the latter is not. Fulghum is resistant to group 2 and thus differentiates this smut group from A 15, A 30, and A 31. Race A 30 was first described by Reed and Stanton (9) and was used by Holton and Rodenhiser (4), who designated it A 15. The evidence accumulated indicates that group 2 may represent a new "Victoria race" of loose smut.

Group 3 is somewhat similar to race A 12, described by Holton and Rodenhiser (4), in that both induced similar reactions on Fulghum. Races 12, 13, 14, 15, and 23, described by Reed (7), also attack Fulghum; therefore, group 3 may be similar to one of these races. Data available did not permit adequate comparisons. Thus, a conclusion concerning the identity of group 3 is not warranted.

Group 4 is pathogenic on certain Red Rustproof varieties (Red Rustproof-14 and Appler). It was thought that this group might be a mixture containing a "Red Rustproof" race similar to race 10, described by Reed (7) and Reed and Stanton (8). However, race 10, with 1 exception (Canadian) in the *Avena sativa* group, attacks only Red Rustproof types, and group 4 vigorously parasitizes Victorgrain 48-93. It is unlikely that group 4 is a mixture that includes a race similar to race 10. This conclusion was reached since this collection has been maintained on Victorgrain 48-93 since 1950, and any race pathogenic only on Red Rustproof types would have been eliminated. In view of these observations, group 4 is apparently different from all of the races described by other workers (4, 7, 8, 10).—Crops Research Division, ARS, U. S. D. A., in cooperation with the Florida Agricultural Experiment Station, Gainesville; Georgia Coastal Plain Experiment Station, Tifton; and Coker's Pedigreed Seed Company, Hartsville, South Carolina.

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EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM CONCENTRATIONS ON THE DEVELOPMENT OF GIBBERELLA STALK- AND ROOT-ROT OF CORN

Paul Thayer and Lansing E. Williams

Respectively, former Research Assistant, Ohio Agricultural Experiment Station, now Assistant Plant Pathologist, Everglades Experiment Station, University of Florida, Belle Glade; and Assistant Professor, Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster.

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SUMMARY

An increase in the concentration of phosphorus in gravel-culture solutions reduced the development of both stalk- and root-rot of corn. Varying the concentration of either nitrogen or potassium had no apparent effect on root-rot. The effect of nitrogen and potassium on stalk-rot development was interrelated so that the effect of each was dependent on the level of the other. An increase in the concentration of nitrogen increased the amount of stalk-rot at the medium and high concentrations but had no effect at the low concentration of potassium. An increase in potassium concentration decreased stalk-rot at low nitrogen concentration, had no effect at medium nitrogen concentration, and increased stalk-rot at high nitrogen concentration.

Field corn is the greatest total dollar income crop in Ohio. Giberella stalk- and root-rot probably account for more loss than any other disease. The per-acre value of field corn, however, is not high enough to permit present-day fungicide treatments for control; therefore investigators must seek methods that are more economical, even though less effective. Manipulation of fertilizer applications has been tested as a possible aid in control that is within the economic limitations.

Foley (2) applied different levels of nitrogen and potassium fertilizers to field plots that were then planted to double-cross hybrids. After pollination, the corn stalks were inoculated with a mixture of *Gibberella zeae* (Schw.), *Diplodia zeae* (Schw.) Lew, and 3 species of *Pythium*. Nitrogen alone increased stalk-rot; potassium alone retarded it. Nitrogen and potassium in equal amounts gave stalk-rot of a severity intermediate to that found when the elements were added separately. Single-cross hybrids growing in field plots treated with varied rates of nitrogen and potassium were inoculated with a mixture of *G. zeae* and *G. fujikuroi* (Saw.) Wt. (7). The results were similar to Foley's, except that the amount of stalk-rot in highly susceptible hybrids was so great that there were no apparent differences from fertilizer treatments. In neither study was stalk-rot affected by phosphate fertilizers.

According to Hoffer (3, p. 59–105), iron compounds accumulate in the nodal tissues of corn plants growing in soils deficient in available potassium. The accumulation of iron results in a breakdown of certain cells in the node. This prevents moving food from leaves to roots, increasing root susceptibility to root-rotting fungi. Hoffer and Carr (4) reported that root-rot was increased by applying aluminum salts to soils; in most instances lime or phosphate fertilizers could reduce it.

Edwards (1) found that in sand cultures the levels of nitrogen, phosphorus, or potassium had no significant effects on seedling blight of corn caused by *G. fujikuroi*. Omission of any of these elements did not affect seedling growth during 2–3 weeks following germination. For the duration of the experiment, minerals in the seed were apparently sufficient to offset any deficiencies in the sand cultures.

In the present study, a mineral-salt gravel-culture technique was used to test the effect of nitrogen, phosphorus, and potassium concentrations on stalk- and root-rot development of corn caused by *Gibberella roseum* (Lk.) Snyd. & Hans. f. *cerealis* Cke. n. comb.

Materials and methods.—The macroelement portion of each solution was 3, 110, or 330 ppm nitrogen; 0, 10, or 100 ppm phosphorus; and 8, 120, or 360 ppm potassium. The concentrations of calcium, sulfur, and magnesium used were as nearly optimum as possible. These minerals were derived from the following 7 salts: KNO_3, $Ca(NO_3)_2$, $CaSO_4$, KH_2PO_4, KCl, $MgSO_4$, and NaH_2PO_4. The 3 concentrations of N, P, and K were used in all possible combinations, making a total of 27 treatments. All treatments were replicated

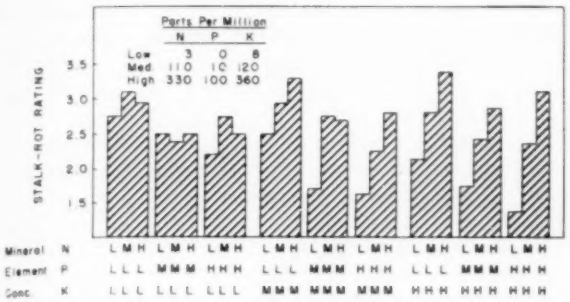
Parts Per Million
N P K
Low 3 0 8
Med 110 10 120
High 330 100 360
STALK-ROT RATING
3.5
3.0
2.5
2.0
1.5
Mineral N
Element P
Conc. K
L M H L M H L M H L M H L M H L M H L M H L M H L M H
L L L M M M H H H L L L M M M H H H L L L M M M H H H
L L L L L L L L L M M M M M M M M M H H H H H H H H H

Fig. 1. Effects of combinations of low, medium, and high concentrations of nitrogen, phosphorus, and potassium on ratings of Gibberella stalk-rot of corn. LSD at 5% = 0.5.

4 times in a random block design, and included 1000 ppm of Meyer's micro-element solution (6) and 10 ppm of a chelated iron.

Corn seed was surface-sterilized with a 1% sodium hypochlorite solution for 10 minutes and planted in 1/8-in.-mesh silica gravel in 3-gal. glazed-clay crocks in the greenhouse. Twice daily a small Cromwell air pump, started and stopped by a Tork electric timer, forced the solutions from mineral salt solution reservoirs into the gravel-cultures. The level to which the solutions were pumped was controlled by venting the air lines. When the pump stopped, the resulting loss of air pressure permitted drainage of the mineral-salt solutions back into the reservoirs. Double-distilled water, obtained by condensation of steam from the heating system, was used in the mineral solutions. An amine, injected into the heating system to prevent rust, resulted in a "carryover" of 3–6 ppm nitrogen into the distilled water; therefore, this amount of nitrogen must be added to that listed above to obtain the total nitrogen content of the solutions. The mineral-salt solutions were replaced with fresh solutions every 3 weeks. The pH of the solutions was adjusted, if necessary, to about 6.2—by adding HCl when only a small amount of chlorine was in the solutions, and by adding H_2SO_4 when the amount of chlorine was large.

Artificial lighting supplied by fluorescent tubes was used to illuminate plants during the winter.

Mass isolates of *G. roseum* f. *cerealis* were obtained from diseased corn stalks in 3 areas of Ohio. Twenty-five single-ascospore isolates were made from these different mass isolates and tested for pathogenicity in replicated field plots. Of the most pathogenic strains, the one producing the most uniform disease rating throughout the test was selected for experimentation.

Results.—*Effect of NPK concentrations on stalk-rot development.*—Two plants were grown in each crock in the stalk-rot experiment. At 9 weeks of age each plant was inoculated in the center of the internode above the ear shank and in the second internode below the ear shank. Stalks were inoculated by the cork-borer fungus-agar disc method described by Williams and Menon (10). Two weeks after inoculation the stalks were split and rated for stalk-rot on the following scale: 1/2, slight necrosis around the point of inoculation; 1, trace to 25% of the inoculated internode rotted; 2, 26–50% rotted; 3, 51–75% rotted; and 4, 76–100% rotted.

TABLE 1.—Main effects of nitrogen, phosphorus, and potassium on Gibberella stalk-rot ratings[a] of corn

Mineral element	Concentration[b] Low	Medium	High	LSD at 5%
Nitrogen	2.0	2.6	2.9	0.7
Phosphorus	2.9	2.4	2.3	0.4
Potassium	2.6	2.5	2.5	NS

[a] Ratings are the mean of 144 inoculations; 1 = trace to 25% rotting of the inoculated internode; 4 = 76–100% rotting of the inoculated internode.

[b] Concentrations in ppm: nitrogen—L = 3, M = 110, H = 330; phosphorus—L = 0, M = 10, H = 100; potassium—L = 8, M = 120, H = 360.

Significant differences in the various combinations of N, P, and K were clearly evident (Fig. 1), but the single effects of N, P, or K were not so evident, nor was the interaction between pairs of elements. Table 1 presents the effect of each element singly. An increase in nitrogen or a decrease in phosphorus resulted in a higher stalk-rot rating, whereas potassium concentration made no difference. Actually, this is not a true representation of the effect of nitrogen or potassium because the action of each element is dependent upon the level of the other, as illustrated in Table 2. Examination of the data revealed: 1) that stalk-rot was reduced by an increase in potassium when nitrogen concentration was low, 2) that potassium concentration made no difference when nitrogen concentration was medium, and 3) that stalk-rot was increased by an increase in potassium when nitrogen concentration was high. When potassium level was low, nitrogen levels made no difference, but at medium and high levels of potassium an increase in nitrogen increased stalk-rot.

Effect of NPK concentrations on root-rot development.—In the root-rot experiment, 6 plants were grown in each crock. When the plants were 3 weeks old, inoculum of *G. roseum* f. *cerealis* was placed in the gravel by an injection-infestation technique described by Schmitthenner and Williams (8). The fungus was increased in liquid culture composed of 0.3% glucose, 0.15% yeast extract, and 0.075% potassium dihydrogen phosphate in distilled water. After 3 days on a shaker, the mycelium was separated from the culture medium by filtration and then resuspended

TABLE 2.—Interaction of nitrogen and potassium on Gibberella stalk-rot ratings[a] of corn

Potassium concentration	Nitrogen concentration[b] Low	Medium	High
Low	2.5	2.7	2.6
Medium	1.9	2.6	2.9
High	1.7	2.5	3.1

LSD at 5% = 0.4

[a] Ratings are the mean of 48 inoculations; 1 = trace to 25% rotting of the inoculated internode; 4 = 76–100% rotting of the inoculated internode.

[b] Concentrations in ppm: nitrogen—L = 3, M = 110, H = 330; potassium—L = 8, M = 120, H = 360.

TABLE 3.—Main effects of nitrogen, phosphorus, and potassium Gibberella root-rot ratings[a] of corn

Mineral element	Concentration[b]			LSD at 5%
	Low	Medium	High	
Nitrogen	2.0	1.9	2.4	NS
Phosphorus	2.4	2.0	1.8	0.4
Potassium	2.3	2.0	1.9	NS

[a] Ratings are the mean of 216 plants; 1 = 5–25% of adventitious roots with lesions, 4 = 76–100% of adventitious roots with lesions.

[b] Concentrations in ppm: nitrogen—L = 3, M = 110, H = 330; phosphorus—L = 0, M = 10, H = 100; potassium—L = 8, M = 120, H = 360.

in an equal volume of distilled water. Fifty ml of this suspension were injected into the gravel under each plant with a 50-ml hypodermic syringe fitted with a tympanol needle. (The tympanol needle, about 5 in. long, closed at the tip, and with 4 openings arranged alternately at ½-in. intervals along the sides, was obtained from the Jensen Salisbury Laboratories, Inc., Kansas City, Mo.)

Two weeks after inoculation the plants were removed and rated for root-rot. Only the adventitious roots were considered in the rating: 0, no root rot; 1, 5–25% of the roots with lesions; 2, 26–50% with lesions; 3, 51–75% with lesions; 4, 76–100% with lesions. The effects of nitrogen, phosphorus, and potassium concentrations were not nearly as pronounced on root-rot as they were on stalk-rot. An increase in the phosphorus concentration decreased root-rot significantly (Table 3). No difference in root-rot could be attributed to the different concentrations of nitrogen or potassium.

Discussion.—Foley (2) and Otto and Everett (7) found that addition of both nitrogen and potassium fertilizers to field plots resulted in stalk-rot development intermediate to that which occurred when each was added separately. In the gravel-culture experiment reported here, an increase in equal proportions of nitrogen and potassium resulted in an increase in stalk-rot. Differences in results might be attributable to one or more of the following factors: 1) a difference in the levels of minerals used, 2) a difference in the time allowed for disease development, and 3) differences in environment. In the field experiments, the nitrogen or potassium in the soil before fertilizer treatments was not determined; therefore no direct comparison can be made between levels of these elements in the soil with that in the gravel-cultures. Nevertheless, the high levels in the gravel-culture solutions were probably higher than any levels in field plots. If, then, the high levels of nitrogen and potassium are eliminated from consideration in Table 2, the results reported here compare more closely with field results. Because of the longer period allowed for stalk-rot development, Foley (2) and Otto and Everett (7) frequently rated stalk-rot extending through more than one internode; in the gravel-culture experiment, stalk-rot was limited to one internode. Fertilizer applications possibly affect the extent of rot differently in nodal versus internodal tissue. Finally, any one of several environmental factors such as availability of water and mineral elements, temperature, light, or type of root growth—which differ in gravel cultures and field plantings—could affect stalk-rot development. Most suspect among environmental factors is an indirect one. Applications of potassium to soils deficient in this mineral frequently reduce lodging of corn even though no stalk-rot occurs. In field plots this would be more of a confounding factor in the reduction of stalk-rot at high potassium levels than it would be in gravel cultures in the greenhouse where no lodging occurred. Adding phosphorus to the gravel culture solutions reduced stalk-rot. This decrease was not reported in field plots (2, 7), probably because the soils were not initially deficient in phosphorus. Phosphorus also reduced the severity of root-rot in the gravel-cultures. The root-rot phase of the Giberrella disease is probably the most important from the standpoint of control, since fairly good evidence has been presented (5, 9) that stalk-rot arises primarily from an extension of root-rot. Thus, maintenance of a high level of phosphorus in the soil should be a worthwhile aid in controlling root- as well as stalk-rot.

On the basis of this study, the mineral balance most consistent with good growth of corn and inhibition of stalk- and root-rot is a medium level of nitrogen and potassium and a high level of phosphorus.—Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, Wooster.

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THE EFFECT OF PH AND MINERALS ON THE HATCHING AND SURVIVAL OF MELOIDOGYNE INCOGNITA INCOGNITA LARVAE

J. R. Loewenberg, T. Sullivan, and M. L. Schuster

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SUMMARY

Studies were made to determine the effect of pH and the mineral composition of the environment on the hatching and survival of *Meloidogyne incognita incognita* larvae. A comparison of several media (Heller's, Knop's, White's, Hildebrandt's et al, Pfeffer's, and Hewitt's) demonstrated the importance of mineral composition of the medium on the emergence and survival of larvae. Heller's solution at a pH of 4.3–4.5 proved superior to the other media. When adjusted to pH 5.7, results were less striking. Heller's solution adjusted to pH 6.5 greatly stimulated the emergence and survival of larvae. Since 1–6-fold strengths of this solution were equally effective, mineral balance appeared to have an important role in emergence.

The conditions influencing the hatching of *Heterodera* larvae have occupied the attention of many nematologists. Information is available on the effect of pH (8), minerals (9), and the "hatching factor" (2) on the emergence of *Heterodera rostochiensis* Wollenweber, but the conditions influencing the hatching of larvae of root-knot nematodes have been neglected. We have found that larvae of *Meloidogyne incognita incognita* (Kofoid & White), though not requiring a hatching factor to emerge, depend greatly on the pH and mineral composition of the environment.

About 50 *M. incognita incognita* egg sacs were picked from tobacco roots and were shaken 4 minutes in a test tube containing 5 ml 10% Purex (a commercial bleach containing 5.25% NaOCl) (10). This treatment broke up the egg sacs so that the eggs dispersed and were surface-sterilized. The egg suspension was then poured into a tube (27 × 200 mm) containing 50 ml sterile water. The eggs were allowed to settle for 20–25 minutes, and were then drawn off with a minimum of liquid. The procedure served to dilute out the bleach and to separate the lighter, smaller eggs, which do not give rise to larvae, from the larger, heavier eggs, which do. Eggs thus obtained were not damaged; in fact, larvae emerged more rapidly from the separated eggs than from eggs contained in egg sacs when both were suspended in Heller's medium. Following dilution with sterile water to the desired concentration, about 400 eggs were pipetted into each test solution (12 ml sterile medium in 60×20-mm Petri dishes). All treatments were made in duplicate.

The suspensions were examined through a dissecting microscope at intervals of several days; all the eggs, live larvae, and dead larvae in several fields were noted and recorded till about 100 observations had been made in each dish. The percentage of live larvae reported below is: live larvae/(eggs + live larvae + dead larvae).

The composition of Heller's medium (3) is: 10 m*M* KCl, 7 m*M* $NaNO_3$, 1 m*M* $MgSO_4$, 0.8 m*M* NaH_2PO_4, 0.6 m*M* $CaCl_2$, and 0.1 m*M* ferric potassium ethylenediaminetetraacetate (5). Modified Heller's solution had an equimolar amount of NaCl substituted for $NaNO_3$. Hewitt's micronutrient solution (4), modified by the addition of KI, was used and has the following composition per liter: $MnSO_4 \cdot H_2O$, 1.690 g; $CuSO_4 \cdot 5H_2O$, 0.249 g; $ZnSO_4 \cdot 7H_2O$, 0.288 g; H_3BO_3, 1.855 g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.037 g; $Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$, 0.316 g; $CoCl_2 \cdot 6H_2O$, 0.024 g; $NiSO_4 \cdot 6H_2O$, 0.026 g; and KI, 0.025 g. One ml of the micronutrient solution was used routinely per liter of medium, and 2 and 3 ml for double- and triple-strength solutions.

A comparison of several media, all having a pH of 4.3–4.5 and containing 0.1 m*M* chelated iron, demonstrated the importance of the mineral composition of the medium on the emergence and survival of *M. incognita incognita* larvae. The superiority of Heller's solution over Knop's (3), White's (3), Hildebrandt's et al (3), Pfeffer's (7) and Hewitt's (4) is apparent (Table 1). Within 2 weeks of the beginning of the experiment the percentage of live larvae in Heller's solution was 75, whereas that in all the other solutions was half as many or less. The results were readily repeated. But when the pH of all these media was initially adjusted to pH 5.7 with KOH, results were less striking.

TABLE 1.—Percentage of eggs of M. incognita incognita hatching, and survival of larvae in various solutions at various times from start of test

	% after various intervals (days)															
	Initial pH 4.3–4.5								Initial pH 5.7							
	2		5		9		13		2		5		9		13	
Solution	Total	Live	Total	Live	Total	Live	Total	Live	Total	Live	Total	Live	Total	Live	Total	Live
Pfeffer's	11	6	19	8	24	11	26	9	21	16	33	26	41	32	48	35
Knop's	20	8	37	11	46	19	28	17	36	31	62	56	69	60	72	61
Hewitt's	15	6	21	7	26	10	19	7	25	19	28	24	54	39	55	33
White's	19	9	32	15	43	23	39	24	20	15	25	25	41	30	49	37
Heller's	37	29	43	26	60	43	76	60	77	35	80	73	77	76	85	77
Hildebrandt's et al	20	10	27	10	30	18	39	15	27	21	46	35	38	35	49	31
Water (control, pH not adjusted)	30	19	38	25	42	27	49	27	29	19	39	25	48	27	45	27

TABLE 2.—Percentage of eggs of M. incognita incognita hatching, and survival of larvae at various times from start of test in triple-strength modified Heller's solution adjusted to different pH levels.

pH before autoclaving	pH after autoclaving	% after various intervals (days)							
		2		7		14		21	
		Total	Live	Total	Live	Total	Live	Total	Live
5.0	5.2	25	20	29	12	49	21	58	30
5.5	5.6	18	11	24	13	54	24	59	23
6.0	6.0	35	30	57	49	77	65	84	73
6.5	6.4	54	50	76	67	90	77	95	82
7.0	6.6	35	26	68	57	87	82	91	83
7.5	6.8	38	28	70	61	85	73	89	72
8.0	7.0	30	21	43	32	81	65	83	65

To test the pH effect, several experiments were made in which modified Heller's solutions of single, double, and triple strength were adjusted with KOH and acetic acid (Table 2). The optimum pH for hatching was about 6.5. On the acetic acid side of this optimum the percentage of live larvae decreased rapidly. Heller's solutions adjusted to pH's above 6.5 were only slightly less effective than the optimum; this was probably because these media approached the optimum pH when autoclaved.

The absence of any of the individual components in Heller's medium could not favor emergence and survival as effectively as the complete mixture. Individual salts were omitted from triple-strength modified Heller's solution in order to establish which salts were essential for emergence and survival. All solutions were adjusted to pH 6.5. The absence of calcium chloride, magnesium sulfate, or chelated iron consistently depressed the number of live larvae to less than one-fourth those present in the complete solution. The omission of sodium or potassium chloride from triple-strength medium also severely depressed emergence and survival, but when either of these two salts was omitted from single-strength solutions, hatching was only slightly lowered. The omission of sodium phosphate depressed hatching only slightly, but the larvae did not live as long in the phosphate-free solutions as they did in the complete medium. The micronutrient elements were necessary for optimum emergence; in their absence, emergence was only slightly depressed. Representative data are given in Table 3.

The concentration at which Heller's solution was most effective in promoting the hatching of *M. incognita incognita* larvae was also investigated. Results were optimum when 1–6-fold concentrations were used; in this range no consistent differences were noted. Although Dropkin et al (1) stressed the importance of osmotic pressure on the hatching of *Meloidogyne* larvae, the osmotic pressure of even 6-fold Heller's solution did not interfere with emergence. More concentrated solutions were definitely toxic, and diluted Heller's solution was clearly less effective.

Since varying the strength of Heller's solution over a 6-fold range had little effect on *M. incognita incognita* emergence, the governing factor is apparently a proper balance of ions rather than the amount of ions involved. This hypothesis is in agreement with the observation that tripling the calcium chloride or magnesium sulfate content of Heller's solution severely depressed emergence; yet, when all the components were tripled, emergence was still maximum. It is conceivable that the increase in hatching that Miller and Stoddard (6) obtained with nabam may have been the result of altering the mineral balance of the nematode eggs, and not a direct metabolic effect. Possibly, such a balance not only controls emergence of the larvae in the soil but also promotes infection of the host plants.

Two differences are apparent between optimum hatching conditions for *Heterodera rostochiensis* and *Meloidogyne incognita incognita*. The optimum pH is 2.0–3.0 for the former and 6.5 for the latter. In a balanced solution, emergence of *H. rostochiensis* is depressed by potassium chloride (2) whereas that of *M. incognita incognita* is stimulated. On the other hand, calcium chloride promotes the emergence of both *H. rostochiensis* (2) and *M. incognita incognita*.—Plant

TABLE 3.—Percentage of eggs of M. incognita incognita hatching, and survival of larvae at various times from start of test in triple-strength modified Heller's solution lacking one component

Lacking component	% after various intervals (days)							
	2		6		10		15	
	Total	Live	Total	Live	Total	Live	Total	Live
Complete solution (control)	26	17	68	58	86	75	90	80
Fe-EDTA	7	2	27	10	34	13	44	17
KCl	14	7	36	27	40	24	42	22
NaCl	20	10	35	21	42	22	50	24
$MgSO_4$	6	0	36	29	39	26	43	22
NaH_2PO_4	18	13	38	30	78	72	84	68
$CaCl_2$	14	5	23	8	27	8	34	9
Micronutrients	13	11	32	20	51	37	74	59
Water (control)	19	8	38	27	49	23	57	30

Pathology Department, University of Nebraska, Lincoln.

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PHYSIOLOGIC SPECIALIZATION IN HELMINTHOSPORIUM TURCICUM

Alice L. Robert

Pathologist, Crops Research Division, ARS, U.S.D.A.
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SUMMARY

Greenhouse tests of 27 single-conidial isolates of *Helminthosporium turcicum* on seedlings of 8 inbred lines of corn, 2 varieties each of sorghum and Sudan grass, and 1 selection of Johnson grass showed that the isolates differed in pathogenicity. Analysis of the mean scores of infection of these isolates on the corn lines indicated there was physiologic specialization, though specificity was not stable enough for use as a criterion in establishing races, as is done with the rusts. Nine different infection types were recognized when the isolates were inoculated on the other hosts: sorghum, Sudan grass, and Johnson grass. Isolates from corn did not infect the sorghum varieties, and those from sorghum did not infect corn. No indication of influence of geographic origin on virulence or avirulence was noticed in the isolates from 18 states of the United States and three foreign countries.

Helminthosporium turcicum Pass. (*Trichometasphaeria turcica* Luttrell) causes a leaf blight of corn (*Zea mays* L.) in most regions of the world where corn is grown. The best method of control is the production of resistant inbred lines. Jenkins and Robert (2) reported that inbred lines of corn differ in resistance to this leaf blight. Further investigations (3, 4, 5) showed that these differences are inherited and controlled by several genes, and that breeding procedures may be used to transfer resistance from resistant to susceptible inbred lines. In studies of the cultural and pathogenic reactions of a number of isolates of *H. turcicum*, the writer (10) found that isolates differ from each other and that the differences may vary from year to year. Jenkins et al (6) later compared the leaf-blight infection of 58 inbred lines for 4–6 years under artificially induced epidemics and determined that seasonal differences constitute a source of variation. The current study reports the physiologic specialization of 27 isolates of *H. turcicum*.

Review of literature.—Elliott and Jenkins (1) observed differences in the severity of infection of some inbred lines of corn by *H. turcicum* in 1942 and 1944. They did not know whether the differences were due to strains of the fungus or to other causes.

Lefebvre and Sherwin (7) reported that 10 isolates of *H. turcicum* from common Sudan grass, Atlas sorghum, Johnson grass, and corn represented 4 distinct races when tested on these hosts. The isolates differed, however, only in their reactions on Sudan grass and sorghum. All were highly virulent on the strain of corn used (Hy × 540). Any changes in virulence after several vegetative transfers of the fungus were not determined.

As early as 1922 Mitra (9) recognized *H. turcicum* on corn and sorghum as 2 separate strains or races in India. Robles (11) identified 2 races of *H. turcicum* on 10 South American strains of corn. He noticed obvious cultural changes in the 2 races as they grew on artificial media, and thought that mutation might have caused the changes in growth and pathogenicity. He concluded that *H. turcicum*, as well as *Helminthosporium carbonum* Ulls. and *Helminthosporium sativum* Pam., King & Bakke, consists of 2 or more parasitic races.

Materials and methods.—Pathogenicity was determined separately for 27 single-conidial isolates of *H. turcicum* on 8 inbred lines of corn, 2 varieties each of sorghum and Sudan grass, and 1 selection of Johnson grass. These isolates came from 18 states of the United States and 3 foreign countries. The following stable inbred lines of corn were used: H548, Mo21A, CI.15, CI.82A, NC34, L97, R4, and 38-11. Atlas and Leoti sorghum and Tift and Sweet Sudan grass were used. The selection of Johnson grass came from rhizomes sent by H. W. Johnson from Mississippi Agricultural Experiment Station, Stoneville, Mississippi. The tests for pathogenicity were made in the greenhouse with plants growing in 4–6-in. pots.

The seedlings were inoculated 3 weeks after planting. To prepare inoculum, a Petri-dish culture of each isolate growing on potato-dextrose agar was mixed for

Table 1.—Mean infection scores (3 experiments) of 27 single-conidial isolates of *Helminthosporium turcicum* on 8 inbred lines of corn.

Isolate	Source	Mean infection scores [a] on various inbred lines						
		Mo21A	CI.15	NC34	L97	R4	38-11	Means
1[b]	Florida	1.0	1.0	1.0	1.0	1.0	1.2	1.0
10	Union of S. Africa	1.2	1.2	1.2	1.0	1.0	1.5	1.1
2[b]	Maryland	1.0	1.0	1.0	1.0	1.0	2.5	1.2
3[c]	Massachusetts	1.2	1.0	1.7	1.0	1.5	1.0	1.2
9	Egypt	1.0	1.2	1.0	1.5	3.3	2.5	1.6
27	New Jersey	2.2	2.3	2.3	1.0	5.8	5.0	2.6
25	Michigan	2.0	4.2	2.7	1.3	6.3	3.7	2.9
14	Egypt	3.7	4.0	4.0	1.5	7.3	7.7	3.6
19	Virginia	4.0	5.7	2.0	2.8	6.2	7.7	3.8
18	Italy	5.2	6.0	3.5	2.3	5.7	7.0	4.0
26	Nebraska	5.0	4.0	4.0	3.0	7.3	7.3	4.0
8	Missouri	3.3	7.3	3.0	3.8	6.8	7.0	4.2
28	North Carolina	4.2	6.2	6.0	2.8	6.7	5.8	4.2
7	Kansas	7.5	3.0	2.5	4.5	6.3	8.0	4.3
11	Delaware	6.7	6.3	3.3	3.2	7.2	6.3	4.4
23	Florida	6.0	6.0	2.7	3.2	7.5	7.2	4.4
12	Union of S. Africa	5.3	7.0	1.3	1.6	9.3	9.7	4.6
15	Texas	4.3	7.7	4.5	3.5	8.3	7.5	4.8
17	Ohio	6.5	7.3	3.7	3.7	8.3	9.0	5.1
13	Union of S. Africa	5.0	7.7	5.2	2.7	9.0	9.0	5.2
20	Wisconsin	5.8	7.3	3.3	3.5	10.3	9.5	5.4
22	Indiana	9.7	6.3	4.7	4.8	8.7	10.0	5.8
16	South Dakota	7.2	9.3	5.7	4.5	9.0	10.0	6.0
24	Iowa	8.0	8.8	3.7	4.3	10.0	10.3	6.1
5	Maryland	8.2	6.8	7.8	5.2	9.7	10.3	6.4
4	Union of S. Africa	9.7	9.5	7.3	7.7	9.7	9.3	7.1
21	Georgia	9.3	8.0	7.5	6.3	10.3	11.0	7.1
Means of inbred lines		4.9	5.4	3.5	3.0	6.8	6.3	

[a] LSD at 1% level for isolates on same inbred line = 2.5.
[b] Isolated from sorghum.
[c] Isolated from Sudan grass.

2 minutes in a Waring blender with about 200 ml of distilled water. The mixture was diluted to 500 ml, and sprayed over the plants until the leaves were well covered. Check pots of each line were sprayed with distilled water. All pots with sprayed plants were covered with a polyethylene plastic sheet on a frame to create a moist chamber. The cover was removed after 24 hours, and the plants were exposed to the regular greenhouse environment. Temperatures were not strictly controlled but were in the range 74–80°F, which favors development of the disease.

Three separate sets of experiments were conducted at intervals of one month. Inocula for the second and third experiments were provided by mass transfers of mycelium and spores from the plates of the first and second cultures, respectively.

Plants were scored for infection in 11 classes of severity at 15–17 days after inoculation. The rating scale was 1–11, from no infection to the most severe infection. Each corn plant was scored separately, and a single collective score was recorded for all plants in each pot of sorghum, Sudan grass, and Johnson grass.

Results.—*Specialization on corn.*—The mean scores of infection of the 27 isolates on 8 inbred lines of corn in the 3 experiments were in the range of 1.0–7.1. The mean scores of all isolates on the inbred lines were in the range of 1.1–6.8 (Table 1). Since there was no significant infection on the inbred lines H548 and and CI.82A by any of the isolates, data on them are omitted from the table. No particular degree of virulence represented any geographic area.

Data on the inbred lines of corn were treated separately from those of other hosts. The inbred lines inoculated with a single isolate were handled as a group. Since the experiments were repeated over an equal period of time, the individual experiments were considered equivalent to replications, and the mean scores of infection were subjected to a split-plot-design analysis of variance. The differences between experiments were significant at the 5% level. This could be accounted for by a change in virulence of the isolates from experiments 1 to 3 or by a uniform and gradual change in environmental conditions. The linear component, which measures any uniform change among experiments, accounted for the major portion of the variability associated with the experiment mean square. Since temperatures were in a favorable range during each experiment and inoculation procedures were fairly uniform, a progressive change in virulence provides the best explanation for the linear trend.

The differences between isolates were highly significant, indicating that they varied in average degree of virulence. There is no valid estimate of error for the interaction of experiments × isolates, but it is interesting that the mean square for the linear component of this interaction is smaller than the unpartitioned interaction mean square.

TABLE 2.—Mean infection scores (3 experiments) of 27 single-conidial isolates of Helminthosporium turcicum on 2 varieties of sorghum, 2 of Sudan grass, and 1 selection of Johnson grass

Isolate	Mean infection scores[a] on Sorghum: Leoti	Sorghum: Atlas	Sudan grass: Sweet	Sudan grass: Tift	Johnson grass	Reaction type
1[b]	R	R	MS	MR	R	I
2[b]	S	MS	S	MS	MS	II
3[c]	R	R	MS	MR	S	III
4, 5, 10, 11, 15, 16, 23	R	R	MS	R	R	IV
7, 9, 22, 27 28	R	R	MR	R	R	V
12, 13, 14, 17, 19, 20, 25, 26	R	R	R	R	R	VI
21	R	R	MR	MR	MR	VII
18, 24	R	R	S	R	R	VIII
8	R	R	S	R	MS	IX

[a] Scores for resistance and susceptibility are: R = 1–2 (resistant); MR = 3–5 (moderately resistant); MS = 6–8 (moderately susceptible); S = 9–11 (susceptible).
[b] Isolated from sorghum.
[c] Isolated from Sudan grass.

The differences between inbred lines were significant at the 1% level. This was expected since, from previous evaluation tests, the lines were known to vary in resistance and susceptibility. The interaction of lines × isolates was highly significant. This indicates that a given isolate did not exhibit the same degree of virulence on all inbred lines, and a given line did not have the same degree of resistance or susceptibility to all isolates. Such results indicate physiologic specialization. The fact that an isolate might change in virulence as a result of sub-culturing from one experiment to another may account for the significance of the interaction of lines × experiments. This type of change has been noted previously (10).

Specialization on sorghum, Sudan grass, and Johnson grass.—Table 2 shows the abilities of the 27 isolates of *Helminthosporium turcicum* to infect 2 varieties of sorghum, 2 of Sudan grass, and 1 selection of Johnson grass. Nine infection types are listed, based on the ability and degree to which isolates infect the 5 hosts. Numbers given to infection types have no significance except to denote that they are different. Isolates 1 and 2 are from sorghum, and represent 2 infection types; isolate 3, from Sudan grass, produces another type. The remaining isolates, from corn, comprise 6 infection types. Infections, except in isolates 13 and 21, were stable during the 3 experiments.

Discussion.—The experiments showed that there are differences in the virulence of 27 single-conidial isolates on 8 lines of corn. In general, the degree of virulence decreased from experiments 1 to 3, as indicated by the linear trend in experiments; however, there were scattered incidents in which virulence increased from experiments 1 to 3 or varied among the 3 experiments. A study of the reactions of varied isolates from 18 states of the United States and 3 foreign countries, Italy, Union of South Africa, and Egypt, showed geographic influence on degree of virulence. It can therefore be assumed that pathogenic strains of the fungus arise independently of location, and that no area can be characterized as having either highly virulent or weakly virulent strains. None of the isolates was highly virulent on all corn lines tested. There is little doubt that races of *H. turcicum* as determined by different inbred lines of corn exist, since the interaction of lines × isolates was highly significant and indicated genetic differences in the isolates. It has not been shown, however, that the host specificity of single-conidial isolates for the 8 inbred lines of corn is stable enough for use as a criterion for separating races of the fungus, as is done with the rusts. No definite conclusions can be reached about the specificity of single-conidial isolates of *H. turcicum* to definite inbred lines of corn, but these tests show that physiologic specialization does exist. The specificity differences prove that careful consideration must be used in separating into groups, through pathogenicity tests, such a widespread and variable fungus when only the conidial stage is used as a source of isolates. Determinations of true races may be reserved for further studies with ascosporic isolates, by which specific genotypes may be established. Since this study was initiated, Luttrell (8) has identified *Trichometasphaeria turcica* Luttrell as the ascosporic stage in the life cycle of *H. turcicum*. This may afford a better opportunity for determining definite races with corn inbred lines.

Specialization of the 27 isolates on 5 other hosts is clear. From the tests 9 infection types were observed. The degrees of infection, except for isolates 13 and 21, were constant throughout the 3 experiments. It is clearly shown that true infection of sorghum isolates on corn and of corn isolates on sorghum did not occur. This agrees with studies and observations of Mitra (9) and Lefebvre and Sherwin (7), who set up definite races of *H. turcicum* with hosts other than corn.—Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

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THE MYCOFLORA OF PEANUTS IN STORAGE

Urban L. Diener

Associate Plant Pathologist.

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SUMMARY

In quantitative determinations of mycoflora, farmer stock peanuts from 26 farm-size bins of several types of construction differed in initial moisture, damage, type of bin, and length of time in storage. High mycofloral counts were associated more often with high initial moistures of peanut kernels than with any other factor. The predominant fungi were several species of the *Aspergillus glaucus* group, *A. tamarii*, and *Penicillium citrinum*. Of the *A. glaucus* group, *A. ruber*, *A. repens*, and *A. restrictus* occurred more frequently and in greater numbers than did *A. chevalieri* and *A. amstelodami*. Other species occurring frequently were *A. candidus*, *Cladosporium* sp., *Torula sacchari*, and *P. funiculosum*. Storage fungi, found in large populations on stored peanuts, probably contribute to deterioration as demonstrated with other kinds of stored seed.

Christensen (3) and Semeniuk (10) recently reviewed seed microbiological research that demonstrated that fungi are involved in the deterioration of stored grains, soybeans, and cottonseed. Studies of the microflora of peanuts in the South have dealt primarily with the fungi associated with concealed damage (7, 14) and seed discoloration (8). Even in varieties highly resistant to concealed damage, the deterioration of peanut seed in storage, as evidenced by loss in viability and processing quality, continues to be a problem in the Southeast. This investigation deals with the species of fungi associated with stored peanut kernels, quantitative measurement of these populations, and their relation to other factors and conditions of storage. A preliminary report has been published (6).

Materials and methods.—The experimental material (obtained through courtesy of the Commodity Credit Corporation and Agricultural Marketing Service, U. S. D. A.) was farmer stock (unshelled and uncleaned) peanuts, *Arachis hypogaea* L. var. Dixie Runner, stored in 26 farm-size bins of the Commodity Credit Corporation at the Wiregrass Substation, Headland, Alabama. Four or more bins (each 625 cu. ft. or greater) were filled during each year of 1952–56 with 4–6 tons of peanuts directly from farmers' fields. The bins drawn on were 7 steel-tube, 8 steel-tight, 1 steel-vent, 6 wood-vent, 3 wood-tight, and 1 farmer-built bin. Steel-tube bins had a perforated steel floor with a vertical tube through the center of the bin and a rotary ventilator on the roof. Steel-tight bins had a solid steel floor with a standard exhaust vent. The steel-vent bin had a perforated steel floor with a standard exhaust vent. Wood-tight bins had a standard wood frame, with siding and floor of tongue-and-groove construction plus composition siding and roofing without ventilation. Wood-vent bins were of wood construction with beveled slatted sides.

Table 1 shows the average grades of all loads placed in each bin. Each load was graded by the Official Method of the USDA, which requires a screen with perforations of 15/64×^{3}4-in. (13).

The bins were sampled on June 5, 1957, by taking two independent 10-lb samples from the surface and two from the middle of the bin. Between June 28 and July 2, when all 26 bins were emptied, a composite sample was collected randomly by taking a cupful of peanuts every 4–5 minutes as the peanuts were moved onto an elevator to load trucks. Subsamples from this composite represented peanuts from all positions within the pile of stored peanuts. With a riffle or divider, a 100-g subsample was randomly selected from each 10-lb and each composite sample of every bin. After being hand-shelled and screened with a 15/64-in. USDA official grading screen, each sample weighed about 50 g and consisted of at least 100 kernels.

The laboratory methods and medium (malt-salt agar) used were as described by Christensen (1) and modified as previously reported (5). After the 50 g of shelled peanuts were ground in a Universal "71" food chopper, a 10-g sample was placed in a sterile 400-ml beaker with 200 ml of sterile tap water. The sample was then homogenized in a Bodine Centrifugal Wet Mill. Under aseptic conditions, dilutions of 1:20, 1:200, 1:2000, and 1:20,000 were prepared by using 10-ml aliquots and 90 ml of water. For thorough dispersion, each dilution was shaken 5 minutes on a Burrell Wrist-Action shaker. A 1-ml aliquot of each dilution was pipetted into each of 3 Petri dishes, and

TABLE 1.—Mold count, moisture content, damage, and sound mature kernels of farmer stock peanuts stored in several types of bins at Headland, Alabama, from 1952 to 1957

Bin No.	Storage			% moisture		% damaged kernels		% sound mature kernels		Mold Count [a]
	Year	Months	Type of bin	In	Out	In	Out	In	Out	(col/g) [b]
1	1952	56	Steel-tube	9.0	5.0	0	7	69	58	4,743
2		56	Steel-tight	10.9	5.4	0	2	62	58	557
3		56	Wood-tight	6.0	5.8	1	2	66	63	33,209
4		56	Steel-tube	6.0	5.6	0	2	66	62	725
5	1953	45	Steel-tube	11.1	5.4	0	2	70	68	165
6		45	Steel-tight	9.8	5.1	0	3	69	61	1,557
7		45	Steel-tube	9.0	5.6	6	10	61	55	20,634
8		45	Wood-tight	8.6	5.7	0	1	68	66	4,480
9	1954	33	Steel-tube	7.6	5.5	0	3	69	66	270
10		33	Steel-tight	9.6	5.5	1	3	67	60	472
11		33	Wood-tight	11.2	5.9	3	3	65	62	117
12		33	Steel-tight	12.4	5.4	1	4	69	63	513
13	1955	21	Steel-tight	15.4	5.9	2	8	70	62	140,167
14		21	Steel-vent	10.0	6.0	1	2	70	67	1,949
15		21	Steel-tight	13.1	5.6	0	2	72	67	73,100
16		21	Wood-vent	12.9	6.3	0	3	70	68	29,683
17		21	Wood-vent	13.4	7.0	1	2	70	69	149,417
18	1956	8	Steel-tight	11.0	5.2	0	2	69	71	29,993
19		8	Wood-vent	6.1	5.8	0	0	70	72	1,282
20		8	Wood-vent	5.8	5.6	1	0	66	73	762
21		8	Home-made	5.8	5.8	1	0	64	70	945
22		8	Steel-tube	5.8	5.0	1	0	64	69	4,033
23		8	Steel-tube	6.0	4.9	0	0	61	66	4,250
24		8	Wood-vent	6.0	6.0	0	1	66	69	1,717
25		8	Steel-tight	6.1	6.1	0	0	67	70	1,387
26		8	Wood-vent	5.6	5.7	1	0	65	72	2,680

[a] Data are means of 4 replications (positional samples).
[b] Mold colonies per gram of peanut kernels.

to each was added 10–12 ml of malt-salt agar cooled to about 45°. The cultures were incubated at 25°C. Mold counts were made with a stereoscopic microscope (10×) at 4–5 days, and again at 8–10 days.

Fungi were transferred to Czapek's solution agar with 3% sucrose for determination of species of *Penicillium*, *Aspergillus*, and other genera. The species of the *Aspergillus glaucus* group were grown on Czapek's agar with 20% sucrose. (The identity of all fungi as to species was confirmed or determined by Dr. C. R. Benjamin, Mycologist, ARS Culture Collection Investigations, Fermentation Laboratory, Northern Utilization Research and Development Division, U. S. D. A., Peoria, Illinois.)

Results.—Table 1 shows the mold count of the positional samples as well as the per cent of kernel moisture, damaged kernels, and sound mature kernels initially and at the completion of the storage experiment. Data are arranged by year and the number of months the peanuts were stored. Type of storage is also indicated.

High mold count was associated with initial kernel moisture contents of 12½% or higher. Most of these high-moisture peanuts were placed in the bins in 1955, a wet curing year. As a result, high mold counts might appear associated with the 1955 storage year or the 21-month period of storage, but the real cause was high initial moisture at bin-filling time. Regardless of initial moisture and bin type, kernel moisture dropped in 30–60 days to 6–7%, considered a safe storage moisture level for unshelled peanuts.

Damage figures in Table 1 refer to the total macroscopic damage to peanut kernels from any cause, such as insects, mechanical injuries, or molds. Neither per cent of initial damage, final damage, nor increase of damage during storage showed a relation to mold count. Mold count was not related to type of bin. Likewise, per cent of sound mature kernels, either initially or at final sampling, and change during storage could not be related to mold count.

Table 2 shows the species of fungi making up the mold counts in Table 1. Counts for 5 species of the *Aspergillus glaucus* group are combined. From these data on frequency, density, and relative density, it

TABLE 2.—Frequency, density, and relative density of the several fungus species constituting the mycoflora of stored Dixie Runner peanuts

Fungus species	Frequency (%)	Density (col/g) [a]	Rel. density (%)
Aspergillus glaucus	100.0	7266	37.1
A. tamarii	84.6	4059	20.7
Penicillium citrinum	100.0	5989	30.6
Cladosporium sp.	100.0	412	2.1
Torula sacchari	80.8	572	2.9
P. funiculosum	38.5	46	0.2
Mucorales	73.1	197	1.0
A. candidus	26.9	452	2.3
P. janthinellum	26.9	71	0.4
Trichothecium roseum	26.9	287	1.5
Aspergillus spp.		18	0.1
Other fungi		223	1.1

[a] Mold colonies per gram of peanut kernels.

TABLE 3.—Frequency, density, and relative density of 5 species of the Aspergillus glaucus group and other fungi of the mycoflora of stored Dixie Runner peanuts

Fungus species	Frequency (%)	Density (col/g) [a]	Rel. density (%)
Aspergillus ruber	100.0	6651	28.4
A. repens	92.3	3016	12.9
A. restrictus	76.9	2052	8.8
A. chevalieri	61.5	473	2.0
A. amstelodami	46.2	32	0.1
A. tamarii	57.7	170	0.7
Penicillium citrinum	84.6	8997	38.4
P. funiculosum	15.4	133	0.6
Torula sacchari	26.9	241	1.0
Cladosporium sp.	57.7	65	0.3
Mucorales	50.0	137	0.6
Trichothecium roseum	23.1	1189	5.0
Other *Aspergillus* and *Penicillium* spp.		36	0.2
Other fungi		231	1.0

[a] Mold colonies per gram of peanut kernels.

became apparent that the predominant species were the *A. glaucus* group, *Penicillium citrinum* Thom, and *A. tamarii* Kita. The species of *Cladosporium* was probably *C. herbarum*, though this identification was not confirmed. *Cladosporium* occurred commonly throughout the study and was the major contaminant resulting from inadequate cleanup and sterilization of equipment between counts. Fungi occurring frequently were *A. candidus* Link ex Fries, *Torula sacchari* Corda, *P. funiculosum* Thom, *P. janthinellum* Biourge, and *Trichothecium roseum* Link ex Fries. The *Mucorales* consisted of *Rhizopus nigricans* Ehr., *Mucor* sp., and occasionally *Cunninghamella* sp. Additional species of *Aspergillus* found were *A. niger* van Tieghem, *A. luchuensis* Inui, *A. terreus* Thom, *A. sydowi* (Bain. & Sart.) Thom & Church, *A. unguis* (Emile-Weil & Gaudin) emend. Thom & Raper, and *A. sulphureus* (Fres.) Thom & Church. Other species of fungi identified were *Paecilomyces varioti* Bainier and *Penicillium lilacinum* Thom. The species of *Fusarium*, *Diplodia*, *Fusidium*, and nonsporulating fungi were not determined. *Bacteria* and *Actinomycetes* were not counted, though they were sometimes present in samples from most bins.

Table 3 shows the proportion of 5 species of the *A. glaucus* group as determined from mold counts of composite samples from each of the 26 bins. On the basis of frequency, density, and relative density, *A. ruber* (*Eurotium rubrum* Brewer), *A. repens* (*E. repens* d By.), and *A. restrictus* G. Smith were the predominant species of this group, though *A. chevalieri* (*E. chevalieri* Mangin) and *A. amstelodami* (*E. amstelodami* Mangin) were frequent. *P. citrinum* was found in high populations and frequencies in the composite samples, sharing dominance with the *A. glaucus* group, as in the positional samples. The decrease in colony density and relative density of *A. tamarii*, however, may have resulted from storing the composite samples at 2°C for 6 months before determinations were made.

Discussion.—In general, high mold count in stored peanuts was associated with high initial kernel moistures. Moisture content of seed in storage is considered (3) one of the critical factors influencing seed invasion by fungi that cause deterioration in stored grains. It thus seems likely that fungi are involved in the deterioration of stored peanuts.

Mold counts were consistently high in peanuts placed in storage with kernel moisture contents above 12½%. Within 3–4 weeks of filling, kernel moisture contents in all bins were about 7% and remained there for 8–56 months. Moisture determinations were made about every 2 months, and were averaged for 7 different positions within the bins. The average moisture of peanuts in all bins went as low as 4.5%, and none ever exceeded 6.8% during the 5-year period. It will be noted in Table 1 that mold counts were high in bins 3, 7, and 18. In bins 3 and 18, the high counts were the result of high mold populations in surface samples, though the peanuts in bin 18 had an initial moisture of 11%. The independent samples in duplicate from the middle of these bins gave low mold counts. The surface count could have been higher because of surface moisture from leakage at a loading port or other cause. High mold count in bin 7 was attributed to high initial damage, resulting from poor curing in a wet year.

The fungi isolated from stored peanuts and identified were in general the same genera and species that literature reviews (3, 10) cited as causing deterioration of grains and other seeds in storage. Investigations with seed of high oil content (4, 9) have shown *Aspergillus glaucus*, *A. flavus*, and *Penicillium* sp. to be the predominant fungi involved in the deterioration of soybeans and cottonseed stored at high moistures. The *A. glaucus* group and *P. citrinum* were the principal molds in stored peanuts. *A. flavus* was not found, but *A. tamarii* occurred frequently and appeared to be important in this study. Tuite and Christensen (2, 11, 12) determined the species of the *A. glaucus* group on barley and wheat and reported *A. repens*, *A. ruber*, *A. amstelodami*, and especially *A. restrictus*, as being involved with germ-damaged or "sick" wheat. In the determination of species of *A. glaucus* from the composite samples from the bins, all four of the above species were identified along with a fifth, *A. chevalieri*. This species was more frequent than *A. amstelodami* in stored peanuts.

Further investigation of these fungi should clarify their role in the deterioration of peanuts in storage.—Department of Botany and Plant Pathology, Agricultural Experiment Station of Auburn University, Auburn, Alabama.

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ASEXUAL VARIANTS OF MELAMPSORA LINI

H. H. Flor

Research plant pathologist, CRD, ARS, U. S. D. A.

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SUMMARY

Pathogenicity genotypes of F_1 cultures of race 22 × race 1 to 25 flax varieties monogenic for resistance were established by crossing and selfing studies. Increases of a urediospore mixture of 4 F_1 cultures on a susceptible variety were screened for variants by dusting on flax varieties highly resistant or immune to the F_1 cultures. The pathogenicity of cultures of each uredium that developed on a resistant variety was determined. Of 129 cultures, 118 were race 22. These could have arisen from asexual nuclear exchange, the reassociation of a "+" race 22 nucleus of one F_1 culture with a "–" race 22 nucleus of another, during hyphal fusion within the susceptible host. All cultures not race 22 varied from one or another of the F_1 cultures in virulence in 1 additional locus for which that culture was heterozygous. The deletion or mutation to the recessive of a dominant gene in the race 1 nucleus of an F_1 urediospore would account for the change in pathogenicity. The 11 spontaneous mutant cultures comprised 7 races, 4 of which were new.

Rust fungi are obligate parasites, highly specialized in their host relationships. Numerous studies have shown that rusts vary, and the extent of variation has been investigated in most species of economic importance. How they vary is less well established; hybridization, mutation, asexual nuclear exchange, and heterocaryosis have been suggested.

Genetic studies of the rusts were made possible by Craigie's (2) discovery of heterothallism. The importance of hybridization in pathogenic variability and the operation of Mendelian laws in the inheritance of pathogenic characters are well established (3, 4, 10, 11).

The roles of asexual nuclear exchange, heterocaryosis, and mutation in pathogenic variability are more difficult to evaluate. Methods for determining the inheritance of pathogenicity of a culture to rust-differentiating varieties have been known for nearly 30 years (13) but have been used effectively only in studies on variability in the flax rust fungus, *Melampsora lini* (Ehrenb.) Lév. (6, 7). Without a genetic basis for pathogenicity there is uncertainty as to which of the possible processes of variability is responsible for a particular variant. Gassner and Straib (8) interpreted the repeated occurrence of the same pathogenic variant in a urediospore culture of *Puccinia glumarum* Schm. f. sp. *tritici* Eriks. & E. Henn. as indicative of mutation, whereas Nelson et al (12) considered the isolation of the same pathogenic variant on two occasions from a mixture of 2 races of *Puccinia graminis* Pers. f. sp. *tritici* (Eriks. & E. Henn.) Guyot as greatly minimizing the possibility that mutation accounted for the increased pathogenicity of the variant.

Fusions of dicaryotic hyphae have been observed in several species of rust fungi (1, 12, 14). It has been suggested (9, 14) that new races may develop through an exchange of nuclei during fusion.

Rust reaction in flax has been explained on the basis of complementary genic systems in host and parasite; for each gene conditioning reaction in the host there is a specific gene for pathogenicity in the parasite (6). This hypothesis implies that the pathogenicity genotype of a rust culture can be determined on differential varieties with single rust-conditioning genes. Such a series of differentials was developed (5). A routine race determination identifies the homozygous recessive genes. The heterozygosity or the homozygosity of the dominant genes is established by selfing the culture.

In the long-cycle heterothallic rusts the dicaryophase is initiated by the transfer of pycniospores from a pycnium of one mating type to a pycnium of the second mating type, and is maintained by conjugate division. The urediospore, which is the repeating phase, is a dicaryotic clone. Barring mutations, all urediospores of an F_1 hybrid culture are genetically identical, and each has 1 nucleus of each parent race. F_1 urediospores of a hybrid of a race of wide pathogenicity and a race of restricted pathogenicity would carry 1

TABLE 1.—Reaction of 16 flax rust differential varieties to races 1 and 22 of Melampsora lini, to F_1 cultures of race 22 × race 1, and to spontaneously derived pathogenic variant races

Differential variety	Reaction* of indicated differential variety to: Parent race		F_1 culture, race 22 × race 1				Spontaneously derived race							
	22	1	A	B	C	D	22	218	250	252	274	275	266	273
Ottawa 770B	S	R	R	R	R	R	S	R	R	R	R	R	R	R
Dakota	S	R	R	S	S	S	S	R	R	R	S	S	S	S
Bombay	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Stewart	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cass	S	R	R	R	R	R	S	R	S	R	R	S	R	S
Koto	S	R	R	R	S	S	S	R	R	R	R	R	S	S
Clay	S	R	R	R	R	R	S	R	R	R	R	R	R	R
Polk	S	R	R	R	R	R	S	R	R	S	R	R	R	R
Birio	S	R	R	R	R	R	S	S	R	R	S	R	S	R
Kenya	S	R	R	R	R	R	S	R	R	R	R	R	R	R
Akmolinsk	S	R	S	S	R	R	S	S	S	S	S	S	R	R
Abyssinian	S	R	R	R	R	R	S	R	R	R	R	R	R	R
Leona	S	R	R	R	R	R	S	R	R	R	R	R	R	R
Wilden	S	R	R	R	R	R	S	S	R	R	S	R	S	R
Victory A	S	R	S	R	R	R	S	S	S	S	R	R	R	R
Bison	S	S	S	S	S	S	S	S	S	S	S	S	S	S

* S = susceptible; R = resistant.

nucleus of each race. Since avirulence is dominant, such a culture would attack only varieties susceptible to both races. If asexual nuclear exchange is a means of pathogenic variation, the virulent race should be recoverable from a mixture of several F_1 cultures of such a hybrid unless the nucleus of the more virulent race is of the same mating type in each F_1 culture.

Material and methods.—Cultures of races 1 and 22 of *M. lini* were crossed and selfed. Race 22 is virulent to all the rust-differentiating varieties of flax except Bombay and Stewart, whereas race 1 is virulent only to Bison and Williston Brown (5).

Pycnial infection with race 22 was sparse. The 4 selfed cultures of race 22 had the pathogenicity of the parent race, a confirmation of results of a previous selfing study (4).

The selfed cultures of race 1 segregated for pathogenicity to the flax varieties Dakota, Koto, Akmolinsk, and Victory A. Although inadequate for a critical test, the 26 selfed cultures of race 1 segregated for pathogenicity into a ratio satisfactorily fitting that expected if avirulence was dominant and the culture of race 1 was heterozygous for pathogenicity in 3 loci. The genes for virulence to Koto and Akmolinsk were allelic or very closely linked, and inherited independently from the genes for virulence to Dakota and Victory A, which also were independently inherited. The χ^2 value for this assumption is 11.21, with P lying between 0.20 and 0.30.

Since race 22 was virulent to all the differential varieties to which race 1 was heterozygous for pathogenicity, and since virulence to each is inherited as a recessive character, the F_1 hybrid cultures were equivalent to test-crosses of a heterozygote to a homozygous recessive. Of 8 F_1 cultures, 5 were virulent to Dakota, and 4 to each of the varieties Koto, Akmolinsk, and Victory A, a good fit to the 1:1 ratio expected. Every culture attacked either Koto or Akmolinsk, and none attacked both varieties. This is expected of allelic or closely linked genes.

Race 22 was homozygous for pathogenicity. Therefore, the haploid race 22 nucleus in each F_1 urediospore carried the recessive gene conditioning virulence to each differential variety shown to be susceptible to parent race 22 in Table 1. Likewise, the race 1 haploid nucleus in each urediospore of the 4 F_1 cultures of race 22 × race 1 listed in Table 1 carried the recessive gene for virulence to the differential varieties susceptible to that culture and the dominant gene for avirulence to the differential varieties resistant to that culture. Thus, the pathogenicity genotype of each of the 4 F_1 cultures of race 22 × race 1 that composed the inoculum was known and formed the basis for interpreting observed variations in pathogenicity.

Each F_1 urediospore culture was increased and tested in a greenhouse section in which no other flax rust was grown. The urediospore mixtures were screened for variants in a range of greenhouses separated from those in which other flax rust work was conducted.

Urediospores of each of the 4 F_1 cultures of race 22 × race 1 were increased on the rust-susceptible variety Bison, and the purity of each culture was confirmed on the flax-rust-differentiating varieties that are monogenic for resistance. About equal quantities of urediospores of each culture were manually mixed and increased on Bison. The resulting increase was diluted with talc, and plants of varieties highly resistant or immune to the F_1 cultures but susceptible to race 22 were inoculated with the spore-talc mixture by means of a cyclone duster. As a rough check on the number of spores screened for variants, plants of Bison were also inoculated. If it is assumed that 1 spore initiated each primary uredium, at least 1 million spores were screened on each of the varieties Ottawa 770B, Cass, Polk, Birio, and Leona.

Each uredium that developed on the varieties resistant to the 4 F_1 hybrid cultures was increased on Bison, and the pathogenicity of the resulting culture on

the 25 flax-rust-differentiating varieties was determined.

Three trials were made for the occurrence of asexual and spontaneous variants. For the first trial, the inoculum was the increase of the 4 F_1 race 22 × race 1 stock cultures. The inocula for the second and third trials were respectively derived from single pustules and from single spores of each culture.

Experimental results.—*Stock cultures.*—The inoculum for this experiment was the urediospore increase of the stock cultures, 5–10 generations from the aecium. In a preliminary test, the first-generation increase of the urediospore mixture on Bison was diluted 1:5 with talc and screened for variants on about 100 plants of each of the varieties Ottawa 770B, Cass, and Birio. Two pustules developed on varieties resistant to the F_1 cultures of race 22 × race 1 that composed the inoculum. A pustule on Cass was race 22. A pustule on Birio was a new race that differed from F_1 cultures C and D in virulence to Birio and Wilden. In inheritance and mutation studies (6, 7) pathogenicity to Birio and Wilden was inherited as a unit.

The second generation of urediospores of the stock culture mixture produced on Bison was diluted 1:5 with talc and screened for variants on about 300 plants of Ottawa 770B, Cass, Polk Birio, and Leona. The leaves of Ottawa 770B, Cass, and Birio, even when heavily inoculated, showed little response to races avirulent to them. In contrast, the heavily inoculated areas of the leaves of Polk and Leona became necrotic. The few pustules that developed in necrotic areas on leaves of Polk and Leona were very small, whereas those near the margins of necrosis were large and vigorous, as were uredia on the varieties that did not show marked necrosis to incompatible races.

All the cultures derived from pustules that developed on Ottawa 770B, Polk, and Leona were race 22. Of 8 cultures from Cass, 7 were race 22 and 1 race 273. The latter differed from F_1 cultures C and D only in virulence to Cass. Of 10 cultures from Birio, 7 were race 22, 2 were race 218, and 1 was race 266. Race 218 differed from F_1 culture A and race 266 differed from F_1 cultures C and D only in virulence to Birio and Wilden.

Single-pustule cultures.—The inoculum for this test was derived from single-pustule increases of each of the 4 F_1 cultures of race 22 × race 1. The first-generation increase of the urediospore mixture, diluted 1:40 with talc was screened for variants on about 200 plants of Ottawa 770B, Cass, Polk, Birio, and Leona. Even at this dilution the leaves of Polk and Leona showed much necrosis.

As in the test with inoculum of stock cultures, all cultures of pustules from Ottawa 770B, Polk, and Leona were race 22. Two cultures of uredia that developed on Cass were race 250, 1 was race 275, and 14 were race 22. Two of the cultures of uredia that developed on Birio were race 274, and 12 were race 22. In this test, 60 of the 65 spontaneous variants were race 22. Each of the other 5 variants differed from one or another of the 4 F_1 race 22 × race 1 cultures in virulence in 1 additional locus for which the F_1 culture was heterozygous for pathogenicity.

Single-spore cultures.—For this test, the F_1 race 22 × race 1 cultures were single-spored. The urediospores of the mixture screened for variants were 3 generations from a single spore. A 1:10 dilution with talc was dusted on 64 plants of each of the varieties used in previous tests.

Of the 16 pustules that developed on varieties either highly resistant or immune to all 4 cultures composing the inoculum, 14 were race 22, 1 was race 250, and 1 was race 252. In this test, as in the preceding ones, every variant was either race 22 or differed from one of the F_1 cultures in virulence in 1 locus for which that culture was heterozygous.

Discussion and conclusions.—An analysis of the genetic basis of pathogenicity in the rusts may explain why the effect of asexual nuclear exchange is observed so infrequently (12). The evidence of asexual nuclear exchange is a change in pathogenicity resulting from the association of a "+" nucleus of one race or culture with the "–" nucleus of another. Obviously, asexual nuclear exchange cannot be detected on varieties to which the mixed races are homozygous for pathogenicity. It can be detected only with difficulty on varieties to which virulence is dominant, since the recombinants cannot be screened on a variety resistant to the mixed races. The variant most likely to be detected would be one resulting from a nuclear exchange between 2 races heterozygous for pathogenicity on a variety to which virulence is recessive. Even then the recessive genes would have to be in compatible nuclei, i.e., in the "+" nucleus of 1 race and in the "–" nucleus of the other. Only if these conditions are met would the recombinant be virulent on a variety resistant to the mixed races.

Asexual nuclear exchange is the simplest explanation for the consistent and frequent recovery of race 22—44 times from the stock cultures, 60 times from the single-pustule cultures, and 14 times from the single-spore cultures—from the urediospore mixtures of 4 F_1 cultures of race 22 × race 1. Race 22 was virulent to 11 varieties not attacked by any one of the F_1 cultures that composed the inoculum. The fact that no pathogenic variant was obtained that is intermediate to those attacking a single additional variety and those attacking 11 additional varieties makes it unlikely that mutations for pathogenicity were responsible for the race 22 variants. Since each of the 4 F_1 cultures carried a race 22 nucleus, it is probable that the race 22 variants originated from the association of a "+" race 22 nucleus of an F_1 culture with a "–" race 22 nucleus of another following hyphal fusion.

Urediospores and mycelial cells of some rust fungi may contain 3 or more nuclei (1, 12). If a cell of an F_1 culture of race 22 × race 1 of *M. lini* had a race 1 "+" nucleus and two race 22 "–" nuclei, the mutation in mating type from "–" to "+" of one of the race 22 nuclei would give that cell both a "–" and a "+" race 22 nucleus and might account for the race 22 variants. That such a process was responsible for the 118 race 22 variants to 11 single gene mutant variants is highly

improbable. Race 22 was not isolated in numerous routine pathogenicity tests to verify the purity of the F_1 race 22 × race 1 cultures. Furthermore, race 22 was not recovered in the spontaneous and X-ray-induced mutation study of culture race 22 × race 1-A, one of the cultures that composed the inocula used in these tests (7). If the race 22 variants were due to mutation in mating type they should have been recovered in much greater frequency following X irradiation than in the current study, in which no mutagen was used.

Each of the 11 variants not race 22 differed from one or another of the F_1 cultures in virulence in 1 additional locus. The spontaneous mutation from the dominant to the recessive in 1 locus for which each F_1 urediospore was heterozygous accounts for this change in pathogenicity. Five of the variants were virulent to Cass, 5 to Birio, and 1 to Polk. This agrees with a study on irradiation-induced mutations with F_1 culture race 22 × race 1-A, in which mutations to virulence occurred with high frequency to Cass and Birio, with intermediate frequency to Polk, and with low frequency to Leona (7). In this, as in the previous study, no mutations to virulence to Ottawa 770B were obtained. The genes most responsive to the mutagenic effects of irradiation appear to be most subject to spontaneous mutation also.

The 11 spontaneous mutant cultures comprised 7 races, of which 3 differed from F_1 culture A, the culture used in the irradiation induced mutation study, by virulence in 1 additional locus, and were described (7). Races having the pathogenicity of the other 4 mutants had not been encountered previously. Races 274 and 275, respectively, differed from F_1 culture B by virulence in the Birio-Wilden and Cass loci. Likewise, races 266 and 273 respectively differed from F_1 cultures C and D by virulence in the Birio-Wilden and Cass loci. Race 250, screened by tests of inoculum derived from single pustules and from single spores, was the only spontaneous mutant obtained in more than 1 test. Not more than 2 cultures of a spontaneous mutant race were obtained in any one test.—ARS, U. S. D. A., Fargo, North Dakota.

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VEIN NECROSIS, ANOTHER SYSTEMICALLY INFECTIOUS STRAIN OF ALFALFA MOSAIC VIRUS IN BEAN

W. J. Zaumeyer and Graciano Patino

Respectively, Principal Pathologist, Crops Research Division, ARS, U. S. D. A.; and Ingeniero Agronomo, Office of Special Studies, Ministry of Agriculture, Mexico, D. F.
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SUMMARY

A virus that produces primary and necrotic symptoms on bean was isolated from infected peas in southern Idaho. The disease it causes, named "vein necrosis," is characterized by necrosis of the veins and veinlets and by irregularly-shaped chlorotic blotches scattered over the leaflets. Necrosis of stems and terminal growing points may also develop. All 26 bean varieties inoculated with the virus were susceptible, though not to the same degree. The virus resembles other strains of the alfalfa mosaic virus in host range and certain physical properties, yet the differences are sufficient to distinguish it as a new strain.

In July, 1956, specimens of diseased pea, *Pisum sativum* L., showing vein clearing and necrosis of the stem were collected in southern Idaho for use in determining the cause of the trouble. The symptoms were similar to the initial ones caused by a new pea streak virus (16) and to those caused by a new strain of tobacco streak virus on peas (8). Diseased pea tissues desiccated for about 4 months produced primary and systemic necrotic symptoms on beans. The studies reported revealed that the virus involved was apparently a new strain of alfalfa mosaic virus. Those studies dealt with the identification of this new isolate.

The disease it causes is named vein necrosis, because of the distinctive symptoms it produces on the inoculated leaves of many bean varieties as a result of veinal infection.

Materials and methods.—The investigations were conducted in the greenhouse at Beltsville, Maryland, at 21–27°C. The virus was maintained on bean and soybean plants. Carborundum powder No. 320 was used as an abrasive in transfers. The inoculations were made by rubbing young fully expanded leaves dusted with carborundum powder with a cheesecloth pad saturated in expressed juice from virus-infected plants.

Results.—*Symptoms and host range.*—Many of the species inoculated with related strains of alfalfa mosaic virus by other investigators (1, 3, 5, 7, 10) were inoculated with the vein necrosis virus. Since the symptoms caused by most of the strains have been described on Bountiful bean, and the symptoms caused by many of the strains have been described on Pinto U.I. 111 bean, the symptomatology of the vein necrosis virus was compared with that of some of the other described strains of the virus on these two varieties. The responses to inoculation of various species and varieties follow:

Phaseolus vulgaris L. (bean). On Bountiful bean, the vein necrosis virus can be distinguished from the alfalfa yellow mosaic virus, which it most closely resembles. Primary necrotic lesions of the vein necrosis virus appear 36–48 hours after inoculation. These are followed by a necrosis of the veins and veinlets of the inoculated leaves, and the formation of irregular blotched chlorotic areas, which may extend over most of the inoculated leaf. These affected leaves develop epinasty and frequently die. The trifoliolate leaflets curl inward and downward and are puckered, and often the veins become necrotic. The petioles and stems of severely infected plants also become necrotic, and the terminal growing points are frequently killed. Relatively young plants are commonly killed by the virus 10–15 days after inoculation.

Alfalfa yellow mosaic virus, on the other hand, usually produces much milder symptoms on Bountiful bean. Relatively small yellow chlorotic spots are formed on the inoculated leaves 4–5 days after inoculation (Fig. 1-J), and mottling of these leaves follows. The trifoliolate leaflets are intensely mottled, and the symptoms somewhat resemble those caused by some strains of bean yellow mosaic virus (Fig. 1-I). The systemic alfalfa mosaic virus reported by Houston and Oswald (3) produces no necrotic lesions on Bountiful. The petioles of the inoculated leaves show internal necrosis, particularly at the nodes. The petioles and veins of the trifoliolate leaves also become necrotic, and complete collapse and death of the growing points results in some cases.

On Pinto U.I. 111 the vein necrosis virus produces local necrotic lesions about 36 hours after inoculation (Fig. 1-L). When numerous on an inoculated leaf the lesions are about 2 mm in diameter, but when sparse some attain a diameter of 5–8 mm. The small lesions are somewhat similar to those produced by the type strain of alfalfa mosaic virus, but large ones are distinctly different. They are reddish-brown with light-cream centers and stippled necrotic borders, and somewhat irregular in outline. On the underside of the leaves, infection is particularly noticeable as a reddish necrosis of the veins and veinlets near the primary lesion. When several lesions coalesce the infected tissues collapse and form a large dead area, and when such areas are numerous the infected leaves die rapidly. Systemic necrosis is produced occasionally. The petioles may become necrotic or the infection may appear only as a slight veinal necrosis of one trifoliolate leaflet. Sometimes the necrosis spreads over the entire leaflet and death results. The nodes show a reddening similar to that produced by the red node virus (11). Alfalfa yellow mosaic virus produces primary necrotic lesions very similar to those produced by the vein necrosis virus, but the systemic symptoms differ considerably. The petioles and pulvini become necrotic, and veinlet necrosis (Fig. 1-F) of the trifoliolate leaves is much more extensive than that caused by the vein necrosis virus. Alfalfa yellow mosaic virus also produces a yellow mottle, downward curling, and malformation of the leaves.

All 26 bean varieties inoculated with the vein necrosis virus were susceptible. With slight variations, the symptoms produced on the following varieties were similar to those produced on Bountiful: Black Valentine, Brittle Wax, Cherokee (Fig. 1-K), Florida Belle, Full Measure, Giant Stringless Green Pod, Landreth Stringless Green Pod, Kentucky Wonder, Kentucky Wonder White, Kentucky Wonder Wax, Light Red Kidney, Logan, Michelite, Plentiful, Rival, Robust, Topcrop, and Unrivaled Wax. The primary necrotic lesions on some varieties were solid necrotic spots, but on others were in the form of ringed patterns (Fig. 1-L). Veinal necrosis (Fig. 1-C) was more severe on some varieties than on others, and systemic necrotic infection and death of the terminal growth and of seedlings were more extensive in a few cases than in others.

The symptoms produced on Blue Lake, Great Northerns U.I. 31 and 123, Sensation Refugee 1066, Stringless Green Refugee (Fig. 1-A,B) and Red Mexican U.I. 34 (Fig. 1-C) were generally similar to those described for Pinto U.I. 111. Only occasionally was systemic infection observed on these varieties. On most of them alfalfa yellow mosaic virus produced a severe systemic mottle and some necrosis of the trifoliolate leaves.

Capsicum frutescens L. (pepper) var. California Wonder. Mild systemic mottle.

Datura stramonium L. (jimsonweed). Mild mottle.

Glycine max (L.) Merr. (soybean). Severe yellow mottle on Ogden (Fig. 1-E) and Jackson varieties, and mild stipple-like mottle on Roanoke and Lee varieties.

Gomphrena globosa L. (globe amaranth). Reddish

local lesions, 2–3 mm in diameter, with white centers.

Lupinus albus L. (white lupine). Local lesions, no systemic infection.

Medicago sativa L. (alfalfa). Mild mottle.

Melilotus alba Desr. (white sweetclover). Mild mottle.

Nicotiana glutinosa L. Systemic mottle and yellow spots on upper leaves that later become necrotic.

Nicotiana rustica L. Mild green mottle and yellow spotting of upper leaves.

Nicotiana tabacum L. var. Samsun (Turkish tobacco). No lesions on inoculated leaves. A mild mottle on upper leaves, becoming masked as plant becomes older. No white stippling of the oak leaf pattern as produced by the type alfalfa mosaic virus.

Petunia hybrida Vilm. (petunia). Large yellow spreading primary lesions; severe systemic mottle.

Phaseolus aconitifolius Jacq. (moth bean). Numerous very small necrotic local lesions.

Phaseolus acutifolius Gray var. *latifolius* Freeman (tepary bean). Numerous small dark-brown local lesions.

Phaseolus aureus Roxb. (mung bean). Large primary necrotic lesions 4 mm in diameter (Fig. 1-D); petiole necrosis and killing of terminal growing point.

Phaseolus coccineus L. (scarlet runner bean). Numerous faint yellow-green and necrotic rings on inoculated leaves; severe petiole necrosis and killing of terminal growth.

Phaseolus mungo L. (urd bean). Spreading primary lesions 4–5 mm in diameter, with light centers. Necrosis of petioles and trifoliolate leaves and killing of terminal growth.

Stizolobium deeringianum Bort. (velvet bean). Mottle and systemic leaf necrosis.

Trifolium pratense L. (red clover). Severe mottle.

Trifolium hybridum L. (alsike clover). Mild mottle.

Trifolium incarnatum L. (crimson clover). Mild mottle.

Vicia faba L. var. *minor* Peterm. (bell bean). Brown primary lesions, systemic stem and leaf necrosis (Fig. 1-G).

Vicia villosa Roth. (hairy vetch). Linear local lesions followed by killing of inoculated leaves.

Vigna sinensis (Torner) Savi (cowpea) vars. Black and Black Eye. Numerous solid reddish-brown local lesions 1–2 mm in diameter (Fig. 1-H); also slight vein necrosis of inoculated leaves. No systemic infection.

Vigna sesquipedalis (L.) Fruwirth (yardlong bean). Numerous brown local lesions.

Other species.—No infection was obtained on *Antirrhinum majus* L. (snapdragon), *Canavalia ensiformis* (L.) DC. (jackbean), *Crotalaria spectabilis* Roth (crotalaria), *Cucumis sativus* L. (cucumber), *Cyamopsis tetragonoloba* (L.) Taub. (guar), *Lupinus angustifolius* L., *Phaseolus lunatus* L. var. Early Thorogreen, *Pisum sativum* L. (pea), *Trifolium subterraneum* L. (sub clover) and *Zinnia elegans* Jacq. (zinnia).

Although the virus here reported was isolated from peas, no record of the name of the infected variety can be found. Nine varieties (Alaska, Dark Seeded Perfection, Early Perfection, Laxton Progress, Little Marvel, Pacemaker, Pride, Prince of Wales, and Wilt Resistant Perfection) were inoculated in the greenhouse, but none became infected. It thus appears that the number of susceptible varieties is limited or that peas are difficult to infect by the rubbing method.

Properties of the virus.—The physical properties of the virus were determined with freshly expressed juice from young systemically infected soybean leaves. Local-lesion production on Pinto U.I. 111 bean was used as a measure of virus activity. For determining the longevity of the virus in dried tissue, infected bean leaves showing local lesions were dried 2–3 days in the open in the laboratory, pulverized, and stored at room temperature in covered Petri dishes. After various periods the leaf tissue was ground with water in a mortar and used to inoculate primary leaves of Pinto U.I. 111 bean.

The virus was inactivated between 62 and 64° C in 10 minutes, and withstood a dilution of 1:4000 but not 1:5000. It was infectious after 30 hours in vitro at 18° C but not after 32 hours. In dry leaf tissue the virus was infectious after 50 days but not after 95 days.

Cross-protection tests.—After plants of Topcrop and Bountiful varieties had become systemically infected with the alfalfa yellow mosaic virus, mottle leaves were inoculated with the vein necrosis virus. Local necrotic lesions failed to develop on these leaves, though they were produced abundantly on leaves of healthy check plants of the same age. These results suggest a relationship between these two viruses. It was not possible to test cross-protection in the reverse direction, because the vein necrosis virus produces only necrotic infection. The micro-precipitin test (12) (performed by Dr. Howard A. Scott) showed the vein necrosis virus to be serologically related to the type strain of alfalfa mosaic virus (AC-108, Catalogue and Registry of the Plant Viruses, 2d Ed., American Type Culture Collection, Washington, D. C.; the anti-serum was produced by Microbiological Research Foundation in collaboration with the American Phytopathological Society Committee on Virus Type Culture Collections).

Discussion.—Since the first report of alfalfa mosaic virus, by Weimer (13) in 1934, seven distinct strains

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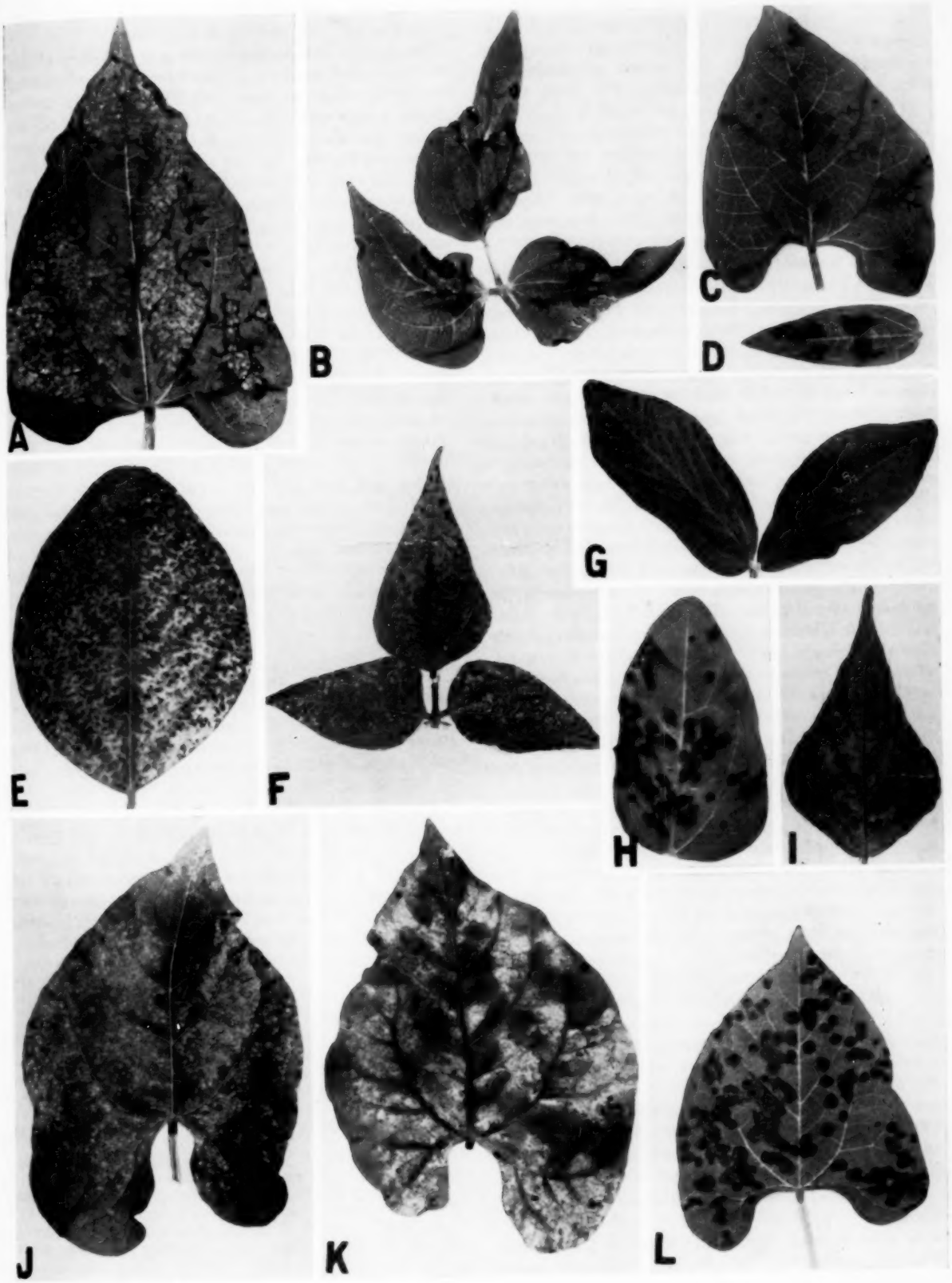
Fig. 1. Symptoms produced by the vein necrosis and alfalfa yellow mosaic viruses on several hosts. Variations in primary necrotic lesions of the vein necrosis virus on **A)** Stringless Green Refugee, **C)** Red Mexican U.I. 34, **K)** Cherokee, and **L)** Pinto U.I. 111 beans. **B,G)** Systemic necrosis produced by vein necrosis virus on Stringless Green Refugee bean and bell bean, respectively. **D,H)** Local necrotic lesions caused by vein necrosis virus on mung bean and Black Eye cowpea, respectively. **E)** Yellow mottle caused by vein necrosis virus on Ogden soybean. **F)** Systemic necrosis and **I)** mottle, produced by alfalfa yellow mosaic virus on Pinto U.I. 111 bean. **J)** Yellow primary spots caused by alfalfa yellow mosaic virus on Bountiful bean.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of the virus have been reported besides the one described here. Because of limited studies of host range and physical properties of the alfalfa viruses reported by Snyder and Rich (9) from celery, by Johnson (4) from tobacco, and by McWhorter (6) from alfalfa and peas, it cannot be determined whether those differ from the other strains reported: potato-calico (2), potato necrosis, pepper mosaic, yellow patch, yellow dot, systemic alfalfa mosaic, and alfalfa yellow mosaic. More strains of alfalfa mosaic virus have been described than of any other legume virus. However, they usually do not damage crop plants seriously.

Though the vein necrosis virus has certain similarities with previously described strains of alfalfa mosaic virus, it can be readily distinguished from them. Since it produces primary necrotic lesions on inoculated leaves and systemic infection on many bean varieties, it appears more closely related to the alfalfa yellow mosaic virus (14, 15) and to the systemic alfalfa mosaic virus (3) than to any of the other strains. Alfalfa yellow mosaic virus also produces both primary and systemic infection on bean, but the symptoms it produces on many varieties, as previously mentioned, differ distinctly from those caused by the vein necrosis virus. The latter causes more severe necrotic symptoms than does alfalfa yellow mosaic virus, but produces no systemic mottle. Vein necrosis virus also differs from alfalfa yellow mosaic virus by infecting pepper, petunia, and red clover, all of which resist the latter, and by not infecting lima bean, pea, snapdragon, and zinnia, which are infected by the alfalfa yellow mosaic virus. Vein necrosis virus on cowpea produces only local necrotic lesions, whereas alfalfa yellow mosaic causes primary necrotic lesions and an intense systemic yellow mottle on this species. The thermal inactivation point is about 3–6 degrees lower for the former than for the latter.

The systemic alfalfa mosaic virus does not produce necrotic lesions on the inoculated leaves of Bountiful bean. This differentiates that virus from the vein necrosis virus. However, the systemic symptoms produced by both viruses on this variety are similar. All the bean varieties inoculated with the systemic alfalfa mosaic virus were seriously infected systemically, but many varieties were seldom infected systemically by the alfalfa necrosis virus. The latter virus produced very mild symptoms on tobacco, whereas the systemic alfalfa mosaic virus produced more intense symptoms on this host. Other host differences are likely, but only a relatively few plant species were inoculated with the systemic alfalfa mosaic virus (3). The thermal inactivation points of the two viruses are similar. The vein necrosis virus lost its infectivity after 32 hours, whereas the systemic alfalfa mosaic virus was still infectious after 72 hours, but not after 96 hours.

On inoculated bean leaves, yellow dot virus (10) only occasionally produces necrotic lesions followed by a very mild systemic mottle and a yellow leaf spotting. These symptoms readily differentiate it from the vein necrosis virus, which produces more severe symptoms on this host. In addition, yellow dot virus infects cucumber and pea; the latter does not.

On Bountiful bean, tuber necrosis (7) and alfalfa N viruses (6) produce primary necrotic lesions very similar to those produced by the type strain of the alfalfa mosaic virus. However, the local lesions on this variety caused by the potato-calico virus (2) somewhat resemble those caused by the vein necrosis virus. Tuber necrosis and potato-calico viruses occasionally become systemic in Bountiful, causing a necrosis of the stem and a dieback of the growing point (7), whereas the alfalfa N virus consistently produces a severe stem necrosis and death of many bean varieties. In contrast, the vein necrosis virus on Bountiful produces primary necrotic lesions that differ considerably in appearance from those produced by the type strain of the virus, and it frequently kills young inoculated plants. Potato-calico and tuber necrosis viruses produce primary necrotic lesions and systemic mottle on pepper and striking systemic mottle symptoms on Datura, tobacco, and cucumber, whereas the vein necrosis virus causes a mild mottle on the first three species and does not infect cucumber. The thermal inactivation points are about 10° lower for the two potato and the alfalfa N viruses than for the vein necrosis virus.

Yellow patch virus (5) and the pepper strain of the alfalfa mosaic virus (1) on beans produce only local lesions very similar to those produced by the type strain. This character helps to distinguish these viruses from the vein necrosis virus. In addition, yellow patch virus infects lima bean, pea, and zinnia; and the pepper virus infects cucumber, pea, and zinnia, all of which are resistant to the vein necrosis virus. On tobacco, both yellow patch and pepper viruses produce more intense systemic symptoms than does the vein necrosis virus.

The necrotic lesions produced on Bountiful bean by the alfalfa local lesion strain of Houston and Oswald (3) and the systemic symptoms produced on some inoculated hosts are similar to those produced by the type strain on these hosts. The thermal inactivation point of the alfalfa local lesion strain is slightly lower than that of the type strain.—Plant Industry Station, Beltsville, Maryland.

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ISOLATION OF VIRUS X FROM "IMMUNE" VARIETIES OF POTATO, SOLANUM TUBEROSUM

A. P. Benson and W. J. Hooker

Respectively Graduate Assistant and Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing. Present address of senior author, State College Station, North Dakota Agricultural College, Fargo.

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SUMMARY

Varieties of potato considered immune to virus X were approach-grafted with scions susceptible to the virus. Scions were either free of virus X at the time of grafting and inoculated with the virus after the graft had become established, or scions were infected with virus X at the time of grafting. Attempts were made to isolate virus X at various time intervals after inoculation. Isolations were made from the complete root system, areas immediately below the graft union, stem sections below the soil line, and necrotic tubers grown on stocks of grafted plants. These tissues were tested by inoculation to *Datura tatula*, which developed a strong systemic mottle when infected with this strain of virus X. The presence of virus X in these varieties was confirmed by serological precipitin tests, synergistic reaction with virus Y on *Nicotiana tabacum*, cross protection with a mild strain of virus X on *D. tatula*, and local lesions on *Gromphrena globosa*.

Virus X was isolated at a very low frequency from all plant parts tested. It was isolated successfully from 11 plants in 20 attempts from USDA seedling clone S.41956, 10 in 27 attempts from the Saco variety, and 10 in 19 attempts from the Tawa variety. Virus X was isolated more frequently and consistently from S.41956 and from Tawa than from Saco, suggesting the possibility of differences in degrees of resistance between "immune" varieties.

In no instance was the virus isolated from leaves of highly resistant varieties grafted with virus-X-carrying scions. It was isolated with some consistency from freshly harvested necrotic tubers that had developed on graft inoculated plants. Although virus X was isolated from the seed pieces, isolations from plants produced from those seed pieces were not successful.

Incubation periods of the virus in *D. tatula* were inconsistent, varying from 5 to 23 days. This and the low frequency of infection suggested extremely low virus concentration in the tissues.

A very high type of resistance to virus X in potato (*Solanum tuberosum* L.) identified by Schultz and Raleigh (17) was later designated as immunity in the seedling S.41956 (15, 16). Attempts to recover the virus from aboveground portions of S.41956 inoculated with virus X by grafting or by mechanical means have been unsuccessful (2, 5, 6, 7, 13, 14, 15, 17). Although the accounts are not detailed, isolations were probably attempted from either leaves or tubers rather than stem or root tissues.

Clinch (5, 6) showed in plants grafted twice with an intermediate scion of resistant S.41956, and with a susceptible stock and top scion that virus X was readily transmitted downward through the intermediate scion to infect the susceptible stock. Upward movement of the virus through an intermediate scion of S.41956 was extremely slow (6) when a susceptible stock was infected with virus X. This suggested that lower stems, roots, and tubers of resistant stocks grafted with a virus-X-carrying scion might yield that virus upon isolation. The purpose of this research was to determine if virus X could be isolated from immune types of potato that had been graft-inoculated with virus X. A preliminary report has been published (4).

Materials and methods. — Isolations were attempted from potato varieties highly resistant to virus X—USDA potato seedling clone S.41956 (15), Saco (1), and Tawa (12). Seed stocks were obtained from a number of reliable sources. They were further identified as being of the immune type of resistance by aerial tuber formation in graft trials (13). The variety S.41956 carries virus S in a latent condition, but this virus was not believed to interfere with the isolation studies since *Datura tatula* L. has not been infected with virus S (3). Isolations were attempted from graft-inoculated stocks. At the time of grafting, scions were either free of or infected with virus X depending upon the particular inoculation treatment. Grafts were wrapped with Sealtex latex bandage.

Scions grafted to resistant stocks were of 3 types: 1) Irish Cobbler or Green Mountain naturally infected with virus X; 2) *D. tatula* plants free of virus X; and 3) susceptible potato plants (*S. tuberosum*) grown from true seed of the Earlaine variety that had been selfed. These seedling plants were maintained free of virus X. Before being grafted, virus-X-free potato scions were demonstrated to be free of virus X by the serological chloroplast agglutination test. Approach-grafted plants were cut 14 days after the initial graft,

TABLE 1.—Isolation of virus X from grafted stocks of "immune" potato clones

Stock	Virus-X-infected scion	Source of tissue for isolation[a]: Root		Subterranean stem		Aboveground stem below graft union	
S.41956	Earlaine selfed	–	–	–	–	+	+
		–	–	–	–	+	–
		+	+	+	–	+	–
		+	+	–	–	+	+
		–	–	–	–	+	+
		+	+	–	–	–	–
		No virus X obtained from 3 plants					
	Datura tatula	+	–	–	–	+	+
		+	–	–	–	–	–
		–	–	–	–	+	+
		+	–	+	–	–	–
		–	–	+	–	–	–
		No virus X obtained from 6 plants					
Saco	Earlaine selfed	–	–	–	–	+	–
		+	+	–	–	–	–
		+	–	–	–	–	–
		+	–	–	–	–	–
		–	–	+	–	–	–
		–	–	–	–	+	–
		+	–	–	–	–	–
		+	–	–	–	–	–
		No virus X obtained from 11 plants					
	Datura tatula	–	–	–	–	+	+
		–	–	+	+	–	–
		No virus X obtained from 6 plants					
Tawa	Earlaine selfed	–	–	–	–	+	+
		–	–	–	–	+	+
		No virus X obtained from 1 plant					
	Datura tatula	+	+	–	–	–	–
		+	+	–	–	–	–
		+	+	–	–	–	–
		+	–	–	–	–	–
		+	+	–	–	–	–
		+	+	–	–	+	+
		+	+	–	–	–	–
		–	–	–	–	+	+
		No virus X obtained from 8 plants					

[a] + denotes successful transfer from potato stock to a single *Datura tatula* plant; – indicates unsuccessful attempt to isolate virus X.

producing a plant with a single scion and a single stock. Virus-X-free Earlaine selfed scions and *D. tatula* scions were mechanically inoculated with isolate X5 (18) 14 days after being grafted.

At intervals following virus X inoculation, attempts were made to isolate the virus from grafted stocks of highly resistant varieties. Plant parts from which isolations were attempted were 1) the complete root system, 2) a 4–6-mm stem section cut from below the graft union and at least 1.5–2.5 mm away from the graft, 3) a stem section near the soil line, and 4) necrotic tubers grown on stocks of grafted plants. Necrotic tubers were occasionally produced on plants grafted with virus-X-carrying scions. Whole tubers or excised necrotic tissues from such tubers were ground in a mortar and pestle and used for inoculating *D. tatula*.

Plant parts to be tested were excised with sterile razor blades and handled with sterile forceps. Portions for isolation were ground using a steamed mortar and pestle. Only enough water was added to the triturated material to provide sufficient inoculum for transfer to an indicator plant. Carborundum, 400 mesh, was mixed with the inoculum.

Tests for virus X were made by mechanical inoculation of 2 *D. tatula* plants. Leaves were sprayed with water, dusted heavily with 400-mesh carborundum, and inoculated with a glass spatula. After rubbing, inoculum was allowed to dry on the leaves, which were later thoroughly washed with water.

In order to evaluate the possibility of chance contamination or of visually unidentifiable incipient infection with virus X in the 2 *Datura* test plants, a third control plant of *D. tatula* was inoculated using one leaf from each of the 2 test plants. Thus, if either test plant had been infected with virus X but not showing symptoms at the time of inoculation with potato juice, symptoms would have been expressed on the control plant. There was no evidence of uncontrolled infection with virus X in any of the *Datura* plants used in these tests.

When virus X was recovered from the tissues of highly resistant plants it was identified as such by each of the following tests: 1) serological chloroplast agglutination test for virus X antiserum (developed against strain X5); 2) synergistic reaction with virus Y in *Nicotiana tabacum* L. variety Havana 38; 3) cross protection in *D. tatula* previously inoculated with a mild strain of virus X, X8 (18); and 4) local-lesion development on *Gomphrena globosa* L.

Plants were grown in sand and watered twice a week with a production-type nutrient solution (an unpublished formula suggested by F. W. Snyder, U.S.D.A., Farm Crops Department, Michigan State University, East Lansing, Michigan) consisting of the following compounds dissolved in 1 gallon of water: 1.39 g $Ca(NO_3)_2 \cdot 4\ H_2O$; 0.69 g NH_4Cl; 0.35 g KH_2PO_4; 0.69 g NH_4PO_4; 0.87 g KNO_3; 139 g $MgSO_4 \cdot 7\ H_2O$; and 1.73 g KCl. Greenhouse temperatures during the period of observation were about 20°C.

Experimental results.—*Mechanical isolations from growing plants.*—Virus X was successfully isolated from 11 of 20 grafted stocks of S.41956 (Table 1). It was obtained from roots, from subterranean stems, and from aboveground stems 1.5–2.5 mm below the graft union. Isolations were slightly more successful from plants grafted with clones of Earlaine selfed inoculated with virus X than from plants grafted with *D. tatula*. Of the 11 plants from which virus X was isolated the virus was obtained from only 1 of the test positions in 7 plants. Virus X was isolated from all 3 test positions of only one grafted plant of S.41956. It was isolated from both roots and stems below the graft union in only 3 instances, and from both roots and subterranean stems in 2 instances. Virus X was isolated in greatest frequency from the area below the graft union, and least frequently from the subterranean stem, suggesting that the virus concentration was considerably higher at the former loca-

tion. Frequency of isolation was higher in roots than in subterranean stems, suggesting that the virus may have accumulated at least to some extent in the roots. Of the 16 successful isolations, only one of the 2 *D. tatula* test plants was infected in 8 isolations and both of the *D. tatula* test plants were infected in the other 8 attempts. Incubation periods in the *D. tatula* test plants ranged from 6 to 23 days. The low frequency of infection of *D. tatula*, and incubation periods longer than normal, suggest extremely low virus concentration in the tissues of S.41956.

Tissues of S.41956 were examined before being ground for inoculum. No macroscopically differentiating symptoms or unusual conditions were associated with tissues from which the virus was successfully isolated. At the time of isolation, roots of S.41956 stocks bearing virus-X-infected scions had become somewhat necrotic.

Isolation of virus X was successful from 10 of 27 grafted Saco plants. Roots of grafted stocks again tended to be slightly necrotic. Virus X was obtained from only 1 location in each of these 10 plants. The virus was apparently present in Saco tissues in very low concentration since both *D. tatula* test plants were infected in only 3 of the 10 successful isolations and incubation periods ranged from 5 to 23 days.

Virus X was isolated in 10 of 19 attempts with grafted plants of the Tawa variety. Results of this series were in general agreement with those from the 2 other varieties. Virus X was recovered from roots in 7 of the 10 successful isolation attempts. In no instance was virus X recovered from the subterranean stem. It was isolated in only 4 instances from the stem portion below the graft union. The virus was isolated from 2 locations in only one plant. The frequency of successful isolations from Tawa and from S.41956 was essentially similar and was higher than that from Saco. When the virus was present in Tawa plants both *D. tatula* test plants were infected in 10 of the 11 isolations. This was much more consistent than was the case with either Saco or S.41956.

Isolations from tubers.—Isolations were attempted from 22 tubers of S.41956 harvested from plants with grafts as previously described. Of these, 6 tubers exhibited an indistinct fleck-type necrosis that was generally distributed throughout the tuber. Usually, only a small percentage of tubers exhibited this limited necrosis. The presence of virus X in tubers was demonstrated by inoculation to *D. tatula* and by subsequent tests for the identity of the virus as previously described. Isolation of virus X was successful from only 1 of 6 necrotic tubers. It was not possible to isolate virus X from the 16 tubers showing no necrosis.

In tests determining resistance in potato to virus A, an extreme necrosis of the cortex was obtained when Green Mountain infected with virus A and virus X were approach-grafted to the Saco variety. Successful isolations of virus X from Saco tubers were made (Table 2) by triturating whole tubers in a mortar and pestle with a small amount of water. Mechanical inoculations were made with a glass spatula. When a virus mottle was produced in the inoculated *D. tatula*, the virus was successively transferred 4 times through that host to eliminate virus A after the method of MacLachlan et al (11). Specific tests for the identification of virus X were applied to all virus cultures following the fourth transfer. Virus X was identified in 9 of 11 isolation attempts (Table 2) indicating at least some survival of virus X in necrotic Saco tubers of plants grafted with a virus-X-infected scion or with a scion doubly infected with viruses X and A.

Necrotic and non-necrotic tubers from S.41956 stocks grafted with Irish Cobbler were stored 3 months and planted in the greenhouse. Plants produced from necrotic tubers were stunted and spindly, whereas plants grown from non-necrotic tubers developed in a normal manner. Isolation of virus X from healthy-appearing and spindly plants was attempted from leaves, stems, roots, and necrotic and non-necrotic seed pieces. Virus X could not be isolated from 4 spindly plants. Virus X was isolated from all 4 necrotic seed pieces that had produced the spindly plants. Furthermore, 4 healthy-appearing plants or their seed pieces yielded no virus X.

Necrotic tubers produced by Tawa stocks that had been grafted with virus-A-carrying Green Mountain were tested similarly. No isolations were successful from non-necrotic tubers or from either spindly or healthy plants. Isolations were successful from 3 of 12 necrotic seed pieces.

In no instance was the virus isolated from tubers of leaf-rubbed mechanically inoculated plants of any immune variety.

Discussion.—Three rather well defined types of resistance to virus X in potato have been reported. This literature has been reviewed by Hooker et al (9). Tolerant types are susceptible, usually exhibiting no symptoms or a mild mottle. Immune types have been described as those varieties in which the virus fails to become established in contrast to hypersensitive types in which top necrosis develops after virus inoculation. Hutton and Wark (10) studied the immune reaction with a seedling similar to S.41956 in its resistance to virus X. Attempts were made to isolate

TABLE 2.—Isolation of virus X from necrotic tubers produced on Saco stocks grafted with virus-X-infected scions, or grafted with scions doubly infected with viruses X and A

	Scion		
Tuber	Variety	Virus present	Isolation of virus X from necrotic tubers[a]
1	Irish Cobbler	X	+
2		X	+
3		X	+
4		X	−
1	Green Mountain	X	+
2		X	+
3		X	−
4		X + A	+
5		X + A	+
6		X + A	+
7		X + A	+

[a] + = virus X isolated; − = virus X not isolated.

virus X from mechanically inoculated leaves of plants with various types of resistance. They considered the immunity of S.41956 to be due to an inactivating system, as evidenced by extremely restricted development of the virus in leaves of the X-immune type as compared to the susceptible types. Others (2) suggested that immunity and hypersensitivity are phases of the same reaction since necrotic spots developed on leaves of S.41956 following grafting to an X-infected stock.

The isolations herein reported suggest that the virus survives, at least to a limited extent, in the tissues of immune varieties. Virus X is either restricted in its development or short lived in these highly resistant varieties. Therefore, under these conditions, the term "immune" should be used advisedly since the virus is at least present in the tissues. The mechanism for resistance has yet to be clearly defined. Furthermore, the possibility exists that the necrotic condition of the tubers may be an indication of a hypersensitive reaction.

For purposes of potato breeding, the varieties S.41956, Saco, and Tawa should still be considered as having a very high degree of resistance useful in the breeding program. It is probable that the virus will not readily move from stored tubers into a growing plant. The term "immune" might well be used with some reservation to designate potato clones with this high type of resistance. Even though the "immune" resistance may in reality be extreme hypersensitivity, there is a need for some term to distinguish between resistance of this type and the generally accepted hypersensitive type of resistance. The use of the term "immune" (16) to describe the type of resistance in S.41956 and derivatives of S.41956 is well established in the literature and there is probably little to be gained by substituting another term until a more complete understanding of the mechanism of resistance is available.

Certain varieties with the "immune" type of resistance may be more resistant than others. This is suggested by greater frequency of successful isolations from the Tawa variety and from S.41956 than from Saco. There was more uniformity of infection of both *D. tatula* test plants with Tawa than with Saco or with S.41956, suggesting differences in degree of virus X survival.

That the virus was not isolated from leaf tissues may be of some significance. The virus is probably transported poorly if at all to the leaves, as suggested by the observations of Clinch (6).

Incubation periods in *D. tatula* varied from 5 to 23 days. Delayed symptom development under these conditions is believed to be a function of low virus concentration (8). With lower concentrations of virus X, the incubation period was extended beyond the normal period required for expression of symptoms in *D. tatula*. When isolations were made from highly resistant varieties, incubation periods ranged from 5 to 23 days, with 5 days probably representing a considerably higher initial concentration of virus in the tissues than a 23-day incubation period. There were no consistent differences between varieties in this respect.

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PHYTOPATHOLOGICAL NOTES

THE EFFECT OF TOBACCO MOSAIC VIRUS ON TOMATO YIELD

P. V. V. Weber

Research by others has shown that infection of tomato plants by TMV (tobacco mosaic virus) usually reduces total yield. The younger the plants at infection, the greater the reduction. Heuberger and Moyer (5) and Heuberger and Norton (6) were the first to relate time of mosaic infection to yields of field-grown tomatoes. They found that mosaic inoculations 4–5 days after field planting reduced total yield of Marglobe about 54%, whereas inoculations two months later reduced it only about 14%. These results have been confirmed in principle (1, 2, 3, 8, 9, 10). McRitchie and Alexander (8) and Walter (10) have also shown that mosaic-tolerant tomato lines may maintain yields despite mosaic infection. In addition, the work of Alexander (2) and McRitchie and Alexander indicates that different TMV strains not only may cause considerably different degrees of total yield reduction in a given variety but also may differentially affect yields of different varieties, including certain mosaic-tolerant lines.

Herein are reported data, obtained in 1957, that indicate the times during harvest when early mosaic infections may reduce yield by statistically and economically significant amounts, thus shedding light on the course of tomato yield reduction by TMV infection, and possibly suggesting control measures. Also presented is an exception to the hypothesis that the earlier the infection the greater the reduction in yield.

Improved Garden State, a tomato variety developed and named by the Campbell Soup Company, was used as a variety ordinarily susceptible to TMV. Preliminary work at this laboratory confirmed Alexander's finding (2) that his variety 80½ (later developed by the Ohio Agricultural Experiment station as W-R Brookston) was less seriously affected by TMV. Two W-R Brookston lines—"A," selected for maximum mosaic resistance, and "B," selected for minimum mosaic resistance—were developed for this experiment. Finally included in the study was a selection from the mosaic-tolerant MStW210-5-2-2, received from Walter (10), Florida Agricultural Experiment Station.

The virus used was Johnson's No. 1 strain of tobacco mosaic virus from H. H. McKinney, received from Doolittle (4), U. S. Department of Agriculture. This virus produced, not necrotic local lesions, but a typical systemic mottle when inoculated to *Nicotiana sylvestris* Speg. & Comes. Likewise, as far as tested, other characteristics of the virus appeared to be typical of the type strain, *Marmor tabaci* var. *vulgare* Holmes (7).

Extracts for inoculations were prepared by expressing juice from diseased tomato plants and filtering through cheesecloth. To reduce fermentation, the extracts were stored as stock inoculum at 5°C under 2% by volume of toluene.

Field planting, in a six-replicate randomized block design, was done on May 29 with a mechanical transplanter. The rows were spaced 10 ft. apart to minimize plant contact between plots. Each plot contained 10 plants 3 ft. apart in the row. About 1 week after planting, each row was mulched with 2 strips of black polyethylene plastic, each 4 ft. wide and .0015 in. thick, placed one on each side of the row so that they overlapped 6–12 in. in the middle of the row, the edges being torn at each plant to permit insertion of the stem. This mulching was to eliminate a need for cultivating close to plants to control weeds, thereby minimizing mechanical mosaic transmission. Hands were washed with soap and water before plants were handled during any operation.

The earlier of 2 sets of inoculations was made on June 7. In all inoculations, a cheesecloth pad was dipped into infectious extract diluted 1:50 with water (carborundum added) and lightly wiped 3 times over the surface of each of 3 leaflets. The early inoculation was on a top and two middle-height leaflets. The later inoculations (1 month after planting) were made on July 2, on 3 middle-height leaflets. One series of randomized plots of each variety was left uninoculated as controls.

The first set of records on the development of mosaic symptoms in individual plants was taken July 12–15, the second set, August 7–9. A visual estimate rating scale was used, from 1.0 for no visible symptoms to 5.0 for very severe mottling. "Mosaic indexes" were computed for each plot by averaging the individual plant ratings for each date.

Pickings were made and yield records taken on August 29, September 6, 16, and 25, and October 4, and yields were computed on the basis of the 5-ft. row spacing in common commercial use, rather than the 10-ft. spacing actually planted.

All data were submitted to the analysis of variance method of statistical analysis, and the least difference between means required for significance (LSD) was computed only when the F value for treatments was significant at the corresponding level.

Although planting was about 2 weeks after the average for the area, and although the season was unusually dry, yields were satisfactory (Table 1). Symptoms were readily apparent in the early-inoculated plots of Improved Garden State on July 1, about 3 weeks after inoculation. By the time of the first mosaic reading, some degree of symptoms was shown by 20% of the uninoculated Improved Garden State plants, 100% of the early-inoculated Improved Garden State series, and 92% of the late-inoculated series. The pattern of infection was generally the same in the W-R Brookston selections, but 10–17 percentage points

TABLE 1.—*Mosaic indexes, and yields from uninoculated and TMV-inoculated tomatoes in a field test at Riverton, New Jersey*

Tomato variety or strains, and inoculation[a]	Mosaic indexes[b,c]		Yield (tons/acre)[c]					
	7/12	8/7	8/29	9/6	9/16	9/25	10/4	Total
Improved Garden State								
Uninoculated	1.2	1.7	3.2	5.0	4.1	2.2	2.0	16.4
Early-inoculated	3.1**	2.6**	2.5**	3.6*	5.3*	2.1	1.5*	15.0
Late-inoculated	**2.1**	2.5**	2.9	3.4**	*4.2	1.8	1.6	13.9*
W-R Brookston, sel. A								
Uninoculated	1.0	1.1	4.9	6.2	7.8	1.8	1.1	19.2
Early-inoculated	1.8**	1.6**	3.5**	6.4	6.6*	2.2	1.4	20.1
Late-inoculated	*1.6**	1.5**	3.7**	5.9	**4.9**	*1.3	1.1	**16.9*
W-R Brookston, sel. B								
Uninoculated	1.1	1.2	2.1	4.8	3.4	1.5	1.5	13.1
Early-inoculated	2.0**	1.7**	1.1**	3.9	4.3	1.6	1.7	12.5
Late-inoculated	**1.7**	1.7**	*1.6*	4.7	4.1	1.6	1.7	13.7
MStW210-5 selection								
Uninoculated	1.0	1.1	1.3	2.8	2.7	2.8	3.1	12.6
Early-inoculated	1.1	1.1	1.0	3.1	2.3	3.1	3.3	12.7
Late-inoculated	1.0	1.1	1.0	2.6	2.2	2.5	*2.8	11.1
LSD 5%	0.2	0.2	0.5	1.1	1.1	0.8	0.5	2.2
1%	0.3	0.2	0.7	1.5	1.4	1.0	0.7	2.9

[a] Early inoculation was made about 9 days, and later inoculation one month, after transplanting to the field.
[b] 1.0–5.0, from no visible symptoms to very severe mottling and distortion.
[c] Asterisks to the right of figure indicate significance rela-tive to the uninoculated entry in each group; asterisks to the left refer to differences between the early- and late-inoculated entries of the group.

lower in the uninoculated series, and 7–9 points lower in the four inoculated series. In the MStW210 series, some degree of symptoms was shown by 17% of inoculated plants and 7% of uninoculated.

By the time of the second reading, the incidence of symptoms in uninoculated Improved Garden State plants had increased from 20 to 70%. Uninoculated entries of the other varieties showed similar increases except that Brookston, selection A, increased from 3 to 21%. All inoculated entries were 97-100% mottled except MStW210, which was about 40% mosaic.

On October 21, six leaf samples, 3 from uninoculated and 3 from early-inoculated plots, were indexed on *Nicotiana glutinosa* L. and *N. sylvestris*. The inoculations from all 3 uninoculated plants and two of the early-inoculated plants resulted in necrotic local lesions on both hosts, and systemic mottling in *N. sylvestris*. The third early-inoculated plant produced only necrotic local lesions on *N. glutinosa* and only systemic mottling on *N. sylvestris*. Thus, 2 weeks after the end of picking, 2 strains of TMV were found in 5 of the sampled plants, but only the inoculated, or a similarly reacting strain, was detected in the sixth.

Mosaic indexes from each of 2 readings, and yield figures from each picking as well as total yields, are shown in Table 1.

The results show that the earlier inoculation significantly reduced the yield of Improved Garden State at the first picking, about 11½ weeks after inoculation, and that the later inoculation was reducing yield significantly by the time of the second picking, 9½ weeks after that inoculation. Similar early yield reductions occurred in the more tolerant W-R Brookston selections, except that, in addition, the late-inoculated entries showed significant yield reductions in the first picking. Thus, such reductions can occur as soon as 8 weeks after inoculation.

In mid-harvest and later pickings, yields tended to be significantly less in the late-inoculated entries than in the early-inoculated, or about the same. Thus, the effect of the early-inoculation was, for the most part, concentrated in the early and mid-harvest pickings rather than in one or more mid-harvest or later pickings. Furthermore, the early-inoculation did not reduce yields by increasingly greater margins of significance as the season progressed.

An exception to the hypothesis that the earlier the infection the greater the total yield reduction, was found in the results from W-R Brookston, Selection A. In this case, total yield was significantly greater at the 1% level in the early-inoculated entry than in the late-inoculated entry.

The yields of the MStW210-5 selection entries were relatively unaffected by the mosaic inoculations.

The results suggest the possibility, particularly if early yield is not highly important, that properly timed early inoculation of tomato varieties reacting as did W-R Brookston, Selection A, with an appropriate mild strain of TMV might be a means of avoiding an economically significant loss in total yield through later infection.—Department of Agricultural Research, Campbell Soup Company, Riverton, New Jersey.

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GROWTH OF APPLE SEEDLINGS IN RELATION TO SOIL TEMPERATURE AND INOCULATION WITH PRATYLENCHUS PENETRANS

W. F. Mai

Root lesion nematodes were shown to be pathogens of possible economic importance when Ark and Thomas (1) described *Anguillulina pratensis* De Man (Goffart) in the roots of poorly growing apple trees in the Sebastopol area of California. When large numbers of these nematodes were added to small apple trees potted in sterilized soil in the greenhouse, root growth was sharply reduced. According to Sher and Allen (4) the nematodes used in these experiments are now classified as *Pratylenchus penetrans* (Cobb). In greenhouse experiments in Australia, D-D treatment of orchard soil naturally infested with *Pratylenchus coffeae* (Zimmerman) resulted in a growth of apple seedlings 9-fold over that of similar seedlings in untreated soil (2). Field observations indicate that *Pratylenchus penetrans* is damaging apple trees in some orchards in New York State.

In a temperature-tank experiment *P. penetrans* significantly reduced the growth of roots and tops of apple seedlings over a wide temperature range. Apple seed of the Cortland variety were sprouted in plastic bags and transferred to 3-in. pots of steam-sterilized soil. About 4000 larvae and adults of *P. penetrans* were added to each of 20 pots. The inoculum was obtained by incubating the orchard grass (*Dactylis glomerata* L.) roots for 2 days in a moist atmosphere at 75°F. *P. penetrans* was the only pathogenic species of nematodes present. Similarly transplanted but uninoculated seedlings served as checks.

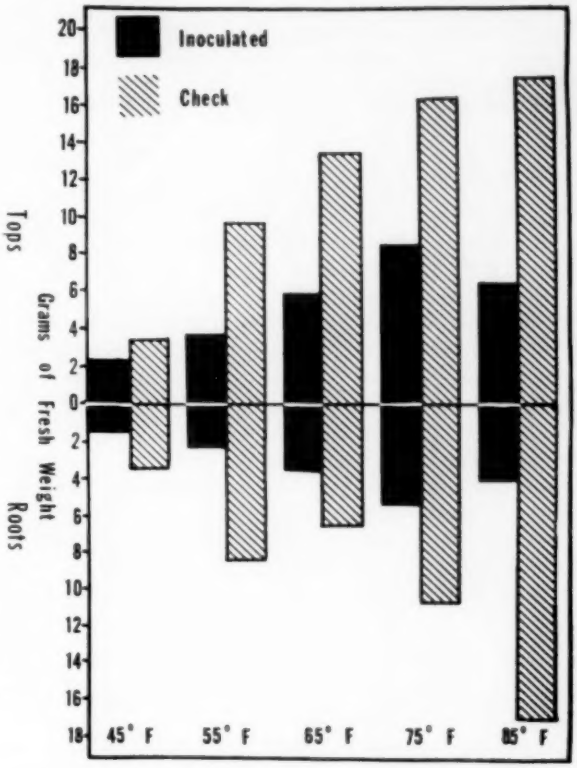

Fig. 1. Plant weight in relation to soil temperature and inoculation with *P. penetrans*.

Four inoculated and 4 uninoculated seedlings were placed in each of 5 soil temperature tanks regulated at 45, 55, 65, 75, and 85°F. After 65 days, the plants were harvested and fresh weights of tops and roots determined (Fig. 1).

Root growth and top growth were influenced significantly by soil temperature. In general, growth of both inoculated and uninoculated plants was correlated directly with temperature. Roots and tops of inoculated plants weighed significantly (P less than .01) less than did those of uninoculated plants. These limited data indicate that greatest reduction in growth, particularly of roots, was at 85°F.

At all temperatures the symptoms on feeder roots of inoculated plants were similar to those caused by *P. penetrans* on the roots of sour cherries (3): 1) necrotic lesions, 2) dead rootlets, 3) over-all dark color of the entire root system, and 4) root systems smaller than those of similar check plants. The root damage was less evident on the roots of plants growing at 45°F than on roots of plants growing at the higher temperatures.—Department of Plant Pathology, Cornell University, Ithaca, New York.

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1. Ark, P. A., and Earl Thomas. 1936. Anguillulina pratensis in relation to root injury of apple and other fruit trees. Phytopathology 26: 1128-1134.

2. Colbran, R. C. 1953. Problems in tree replacement. 1. The root-lesion nematode Pratylenchus coffeae Zimmerman as a factor in the growth of replant trees in apple orchards. Australian J. Agr. Research 4: 384-389.

3. Parker, K. G., and W. F. Mai. 1956. Damage to tree fruits by root lesion nematodes. Plant Disease Reptr. 40: 694-699.

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BOOK LISTINGS

PLANT PATHOLOGY: PROBLEMS AND PROGRESS, 1908-1958. Edited by C. S. Holton, G. W. Fisher, R. W. Fulton, Helen Hart, and S. E. A. McCallan. The University of Wisconsin Press, Madison, Wisconsin. 588 p. Price $8.50.

This Golden Jubilee Volume contains addresses and papers presented at a meeting commemorating the fiftieth anniversary of the American Phytopathological Society. Part I consists of seven major addresses describing the historical and developmental aspects of plant pathology. The proceedings of nine symposia and a 12-page index occupy the rest of the volume. The subjects covered by the symposia were: Physiology of parasitism, genetic approach to elucidation of mechanisms governing pathogenesis and disease resistance, fungicides, chemistry of fungicides, soil microbiology and root-disease fungi, concepts and problems of nematology, structure of viruses, multiplication of plant viruses, and epidemiology of plant diseases.

Cummins, George B. 1959. **Illustrated genera of rust fungi.** Burgess Publishing Co., Minneapolis, Minnesota. 131 p. Price $4.50.

This manual presents keys, descriptions, and illustrations of the genera of the rust fungi of the world. In addition, it contains general references, regional lists, partial list of synonyms, a glossary, and an index to the genera. The keys are organized in a very simple fashion, without the use of families. The genera are distributed through 10 sections, both as to key and the text. Some genera are keyed out in more than one section. Only fungus characters, mainly those of teliospores, are used in the keys. Illustrations consist of photographs, schematic drawings, and camera lucida drawings. This manual should prove very useful to students and practicing plant pathologists alike.

Goodey, J. Basil, Mary T. Franklin, and David J. Hooper. **Supplement to the Nematode Parasites of Plants Catalogued Under Their Hosts 1955-1958** (2nd edition, August 17, 1959). Copies can be obtained from the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England. Price 7s. 6d.

This publication is a further addition to the original compilation, made by Dr. T. Goodey in 1940, and revised and brought up to date by J. Basil Goodey and Mary T. Franklin in 1956. The authors have adhered to the style of the 1956 revision, which gives the scientific names of nematodes and their hosts, the latter being supplemented by common names in many instances. Authorities for host records, and a comprehensive list of references, are presented. The three publications represent the most complete listing available of nematode host plants.

Poehlman, John Milton. 1959. **Breeding field crops.** Henry Holt and Co. New York. 427 p. Price not given.

This book, designed primarily for the introductory course in crop breeding, contains many excellent photographs and line drawings by Hanna T. Croasdale, Dartmouth College. The first chapter describes the plant breeder and his work. The next four chapters deal with reproduction in crop plants, genetics in relation to plant breeding, and the methods and techniques employed. The last 13 chapters are devoted to breeding of specific field crop plants. A glossary of plant-breeding terms is provided.

WALLACE, JAMES M. ed. 1959. **Citrus virus diseases.** University of California Division of Agricultural Sciences, Berkeley. 243 p. Price $3.50.

This book, dedicated to the memory of Prof. H. S. Fawcett, contains proceedings of the Conference on Citrus Virus Diseases, held at Riverside, California, November 18-22, 1957, on the fiftieth anniversary of the founding of the Citrus Experiment Station. Thirty-five papers cover progress and outstanding achievements in the field. Following reports on the economic importance of citrus viruses, psorosis, and viruses in the Mediterranean area, are nine papers on tristeza in Africa and North and South America. The next chapters cover stubborn disease, xyloporosis and cachexia, exocortis (five chapters), and vein enation. The final thirteen chapters deal with transmission of a growth-retarding factor, bud-union disorder, symptomless carriers, nucellar embryony, rootstock in control of tristeza, indexing for viruses, maintaining virus-free stock, research in Egypt, virus-free propagation sources, budwood certification programs in Texas, Florida, and Arizona.

GRAY, WILLIAM D. 1959. **The relation of fungi to human affairs.** Henry Holt and Co., New York. 510 p. Price $8.50.

This volume, designed primarily for courses in industrial mycology, begins with a brief elementary discussion of the morphology, classification, identification, and methods of culturing fungi. Part I contains 19 chapters dealing with the beneficial functions of fungi in destruction of organic waste, processing of food for man and animals, and production of industrial chemicals and medicines. Part II, containing 6 chapters, describes the harmful role fungi play in the destruction of timber, and timber production, food spoilage, and as agents of diseases in plants and animals. Literature cited is listed at the end of each chapter.